

Clinical Exome Sequencing Analysis – Carrier Screening

Patient name	: Mr. XXX	Mrs. YYY
Gender/ Age	: Male/ 42 years	Female/ 30 years
PIN	: XX	XX
Sample no	: XX	XX
Specimen	: Peripheral blood	Peripheral blood
Sample collection date	: XX	XX
Sample receipt date	: XX	XX
Report date	: XX	XX
Referring clinician	: XX	
Hospital/Clinic	: XX	

Clinical history

Mr. XXX and Mrs. YYY are a non-consanguineous couple and they presented with congenital hearing loss and mutism. Mrs. YYY was diagnosed with bilateral tubal block and Mr. XXX was diagnosed with Hypothyroidism and Asthenoteratozoospermia. They have a history of 6 failed OI + TI. Mrs. YYY's brother also has congenital hearing loss and mutism. Mr. XXX and Mrs. YYY have been evaluated for carrier status of pathogenic variations.

Results

Mrs. YYY is found to be affected with likely pathogenic variants in the *SLC26A4* genes (c.1614+1G>A) and (p.Arg581AlafsTer26).

Mr. XXX is found to be affected with uncertain significant variants in the *TG* genes (p.Gly2348Arg) and (p.Thr2273Met).

Mr. XXX is found to be carrier of likely pathogenic variants in the *FAM161A* gene (p.Leu123Ter) and *CHST14* gene (p.Tyr293MetfsTer141).

Mrs. XXX is found to be a carrier of uncertain significant variant in the *SYNE1* gene (p.Leu3553Pro).

Mrs. YYY is found to be a carrier of uncertain significant variant in the *SYNE1* gene (p.Lys6435Asn).

List of variants identified related to the phenotype:

Disease	Mr. XXX	Mrs. YYY
<p>Deafness, autosomal recessive 4, with enlarged vestibular aqueduct (OMIM#600791) Pendred syndrome (OMIM#274600)</p> <p>Mode of inheritance: AR Reproductive Risk Score: 1 in 1024784 Cumulative carrier frequency:1/82 Genetic Prevalence: 1 / 26533</p>	<p>NON - CARRIER</p>	<p>Gene: <i>SLC26A4</i> Exon 16, c.1741_1742delAG p. Arg581AlafsTer26 Heterozygous Classification: Likely Pathogenic</p> <p>Gene: <i>SLC26A4</i> Intron 14, c.1614+1G>A 5' splice site Heterozygous Classification: Likely Pathogenic</p>
<p>Thyroid dyshormonogenesis 3 (OMIM#274700)</p> <p>Mode of inheritance: AR Cumulative carrier frequency:1/314 Genetic Prevalence: 1 / 393785</p>	<p>Gene: <i>TG</i> Exon 41, c.7042G>A p. Gly2348Arg Heterozygous Classification: Uncertain Significance</p> <p>Gene: <i>TG</i> Exon 39, c.6818C>T p. Thr2273Met Heterozygous Classification: Uncertain Significance</p>	<p>NON - CARRIER</p>

List of significant carrier variants identified:

Disease	Mr. XXX	Mrs. YYY
<p>Retinitis pigmentosa 28 (OMIM#606068)</p> <p>Mode of inheritance: AR Cumulative carrier frequency:1/598 Genetic Prevalence: 1 / 1429074</p>	<p>CARRIER</p> <p>Gene: <i>FAM161A</i> Exon 2, c.368delT p. Leu123Ter, Heterozygous Classification: Likely Pathogenic</p>	<p>NON - CARRIER</p>
<p>Ehlers-Danlos syndrome, musculocontractural type 1 (OMIM#601776)</p> <p>Mode of inheritance: AR Cumulative carrier frequency:1/4614 Genetic Prevalence: 1 / 85134336</p>	<p>CARRIER</p> <p>Gene: <i>CHST14</i> Exon 1, c.876delC p. Tyr293MetfsTer141 Heterozygous Classification: Likely Pathogenic</p>	<p>NON - CARRIER</p>

List of uncertain significant carrier variants identified:

Disease	Mr. XXX	Mrs. YYY
Arthrogryposis multiplex congenita 3, myogenic type (OMIM#618484) Spinocerebellar ataxia, autosomal recessive 8 (OMIM#610743) Mode of inheritance: AR	CARRIER Gene: <i>SYNE1</i> Exon 67, c.10658T>C p. Leu3553Pro, Heterozygous Classification: Uncertain Significance	CARRIER Gene: <i>SYNE1</i> Exon 104, c.19305G>C p. Lys6435Asn Heterozygous Classification: Uncertain Significance

*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 variant identified related to the phenotype in Mrs. YYY Likely compound heterozygous variants identified in *SLC26A4* gene

Variant 1: *SLC26A4*: c.1741_1742delAG

Variant summary: A heterozygous two base pair deletion in exon 16 of the *SLC26A4* gene (chr7:g.107701132delAG, NM_000441.2, Depth: 84x) that results in a frameshift and premature truncation of the protein 26 amino acids downstream to codon 581 (p. Arg581AlafsTer26) was detected. This variant is a frameshift variant which occurs in an exon of *SLC26A4* 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.

Clinical evidence: This variant has been previously classified as pathogenic in ClinVar database [3].

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

Variant classification: Based on the evidence, this variant is classified as likely pathogenic variant. **In this view, clinical correlation and familial segregation analysis are strongly recommended to establish the significance of the finding. If the results do not correlate, additional testing may be considered based on the phenotype observed.**

Variant 2: *SLC26A4*: c.1614+1G>A

Variant summary: A heterozygous 5' splice site variation in intron 14 of the *SLC26A4* gene (chr7:g.107698112G>A, NM_000441.2, Depth: 88x) that affects the invariant GT donor splice site of exon 14 (c.1656+1G>A) was detected.

Population frequency: This variant has minor allele frequency of 0.0013% in gnomAD database and has not been reported in 1000 genomes databases.

Clinical and Literature evidence: This variant has been previously classified as pathogenic in ClinVar database [4]. This variant has been previously reported in patients affected with hearing loss in compound heterozygous state [5].

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

Variant classification: Based on the evidence, this variant is classified as likely pathogenic variant. **In this view, clinical correlation and familial segregation analysis are strongly recommended to establish the significance of the finding. If the results do not correlate, additional testing may be considered based on the phenotype observed.**

OMIM phenotype: Deafness, autosomal recessive 4, with enlarged vestibular aqueduct (OMIM#600791) and Pendred syndrome (OMIM#274600) is caused by homozygous or compound heterozygous mutation in the *SLC26A4* gene (OMIM*605646). DFNB4 with enlarged vestibular aqueduct is characterized by pre- or perilingual onset of sensorineural or mixed hearing loss, which may be fluctuating or progressive. The hearing loss is associated with temporal bone abnormalities, most commonly enlargement of the vestibular aqueduct, but it can also include the more severe Mondini dysplasia, a complex malformation in which the normal cochlear spiral of 2.5 turns is replaced by a hypoplastic coil of 1.5 turns. Pendred syndrome, the most common syndromal form of deafness, is an autosomal recessive disorder associated with developmental abnormalities of the cochlea, sensorineural hearing loss, and diffuse thyroid enlargement. This disease follows autosomal recessive pattern of inheritance [2].

The variants are reported to be in likely compound heterozygous state. These likely compound heterozygous variants are strongly recommended to confirm the cis or trans status by parental segregation analysis.

Interpretation for the uncertain significant variant identified related to the phenotype in Mr. XXX
Likely compound heterozygous variants identified in *TG* gene

Variant 1: *TG*: c.6818C>T

Variant summary: A heterozygous missense variation in exon 39 of the *TG* gene (chr8:g.133019637C>T, NM_003235.5, Depth: 91x) that results in the amino acid substitution of Methionine for Threonine at codon 2273 (p. Thr2273Met) was detected.

Population frequency: This variant has minor allele frequency of 0.003% in gnomAD database and has not been reported in 1000 genomes databases.

In-silico prediction: The *in-silico* predictions of the variant are damaging by SIFT.

Variant classification: Based on the evidence, this variant has been classified as uncertain significance variant. **In this view, clinical correlation and familial segregation analysis are strongly recommended to establish the significance of the finding. If the results do not correlate, additional testing may be considered based on the phenotype observed.**

Variant 2: *TG*: c.7042G>A

Variant summary: A heterozygous missense variation in exon 41 of the *TG* gene (chr8:g.133029826G>A, NM_003235.5, Depth: 99x) that results in the amino acid substitution of Arginine for Glycine at codon 2348 (p. Gly2348Arg) was detected.

Population frequency: This variant has minor allele frequency of 0.002% in gnomAD database and has not been reported in 1000 genomes databases.

Clinical evidence: This variant has been previously classified as uncertain significance in ClinVar database [6].

Variant classification: Based on the evidence, this variant is classified as uncertain significance variant. **In this view, clinical correlation and familial segregation analysis are strongly recommended to establish the significance of the finding. If the results do not correlate, additional testing may be considered based on the phenotype observed.**

OMIM phenotype: Thyroid dysmorphogenesis 3 (OMIM#274700) is caused by homozygous or compound heterozygous mutation in the *TG* gene (OMIM*188450). Thyroid dysmorphogenesis caused by mutations in the thyroglobulin (*TG*) gene. This form of thyroid dysmorphogenesis has an estimated prevalence of one in 100,000 newborns. Inherited in an autosomal recessive manner, the disorder in the majority of patients causes large goiters of elastic and soft consistency. Although the degree of thyroid dysfunction varies considerably among patients with defective *TG* synthesis, patients usually have a relatively high serum free T3 concentration with disproportionately low free T4 level. This disease follows autosomal recessive pattern of inheritance [2].

The variants are reported to be in likely compound heterozygous state. These likely compound heterozygous variants are strongly recommended to confirm the cis or trans status by parental segregation analysis.

Interpretation for the significant carrier variants identified in Mr. XXX

FAM161A: c.368delT

Variant summary: A heterozygous single base pair deletion in exon 2 of the *FAM161A* gene (chr2:g.61842176delA, NM_001201543.2, Depth: 85x) that results in a termination of the protein at codon 123 (p. Leu123Ter) was detected. This variant is a frameshift variant which occurs in an exon of *FAM161A* 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.

Clinical evidence: This variant has been previously classified as likely pathogenic in ClinVar database [7].

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

OMIM phenotype: Retinitis pigmentosa 28 (OMIM#606068) is caused by homozygous or compound heterozygous mutation in the *FAM161A* gene (OMIM*613596). This disease follows autosomal recessive pattern of inheritance [2].

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

CHST14: c.876del

Variant summary: A heterozygous single base pair deletion in exon 1 of the *CHST14* gene (chr15:g.40472089delC, NM_130468.4, Depth: 85x) that results in a frameshift and premature truncation of the protein 141 amino acids downstream to codon 293 (p. Tyr293MetfsTer141) was detected. This variant is a frameshift variant which occurs in an exon of *CHST14* 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 tool.

OMIM phenotype: Ehlers-Danlos syndrome, musculocontractural type 1 (OMIM#601776) is caused by homozygous or compound heterozygous mutation in the *CHST14* gene (OMIM*608429). This disease follows autosomal recessive pattern of inheritance [2].

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

Interpretation for the uncertain significant carrier variant identified in Mr. XXX

SYNE1: c.10658T>C

Variant summary: A heterozygous missense variation in exon 67 of the *SYNE1* gene (chr6:g.152354927A>G, NM_182961.4, Depth: 96x) that results in the amino acid substitution of Proline for Leucine at codon 3553 (p. Leu3553Pro) was detected.

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

Clinical evidence: This variant has been previously classified as uncertain significance in ClinVar database [8].

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

OMIM phenotype: Arthrogryposis multiplex congenita 3, myogenic type (OMIM#618484) and Spinocerebellar ataxia, autosomal recessive 8(OMIM#610743) are caused by homozygous or compound heterozygous

mutation in the *SYNE1* gene (OMIM*608441). These disease follows autosomal recessive pattern of inheritance [2].

Variant classification: Based on the evidence, this variant is classified as uncertain significance variant.

Interpretation for the uncertain significant carrier variants identified in Mrs. YYY

***SYNE1*: c.19305G>C**

Variant summary: A heterozygous missense variation in exon 104 of the *SYNE1* gene (chr6:g.152255045C>G, NM_182961.4, Depth: 88x) that results in the amino acid substitution of Asparagine for Lysine at codon 6435 (p. Lys6435Asn) was detected.

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

Clinical evidence: This variant has been previously classified as uncertain significance in ClinVar database [9].

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

OMIM phenotype: Arthrogryposis multiplex congenita 3, myogenic type (OMIM#618484) and Spinocerebellar ataxia, autosomal recessive 8(OMIM#610743) are caused by homozygous or compound heterozygous mutation in the *SYNE1* gene (OMIM*608441). These disease follows autosomal recessive pattern of inheritance [2].

Variant classification: Based on the evidence, this variant is classified as uncertain significance variant.

Additional Variant(s)

The additional variants identified which are significant but may not be related to patient's phenotype. Phenotype – genotype correlation is recommended

List of additional variants identified:

Disease	Mr. XXX	Mrs. YYY
Saul-Wilson syndrome (OMIM#618150) Mode of inheritance: AD	Gene: <i>COG4</i> Exon 9, c.1106G>T p.Arg369Leu Heterozygous Classification: Uncertain Significance	NON - CARRIER

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 targeted gene capture using a custom 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 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	8.61Gb	8.37Gb
Data ≥ Q30	92.63%	92.63%

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 [10].

References

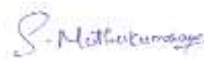
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7. <https://www.ncbi.nlm.nih.gov/clinvar/variation/VCV001443212.5>
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9. <https://www.ncbi.nlm.nih.gov/clinvar/variation/VCV002054509.2>
10. Kalia S.S. et al., Recommendations for reporting of secondary 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.

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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, AHCY, AHSX, 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, ARFGEF2, ARHGDI, ARHGEF6, ARL2BP, ARL6, ARL6IP1, ASPM, ATCAY, ATG5, ATP2A1, ATP2B3, ATP6AP2, ATP6V1A, 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, CA5A, 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, CNNM2, 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, CTH, 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, 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3PXD2B,SH3TC2,SP7,ABCD3,ALDH4A1,CD81,INPP5E,VPS13C,WNT1,FGD1,GOSR2,IQCE,MDH2,MIPEP,PROM1,PTPRO,SBF1,ATP13A2,COL27A1,DCDC2,D
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14,BRCA1,CC2D2A,CFI,GIPC3,CLKB1,OPA1,SCP2,SPAG1,TAPBP,TBCK,UBA5,CLCF1,HFM1,MAGED2,PTPN22,SIPA1L3,SLC01B1,TALDO1,THOC2,UBE2A,AP
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