Trio-Whole Exome Sequencing Analysis

Patient name	**: Baby. ZZZ**	**PIN**	**: XX**
Gender/ Age	**: Female / 4 Years**	**Sample number**	**: XX**
Referring clinician	**: XX**	**Sample collection date**	**: XX**
Hospital/Clinic	**: XX**	**Sample receipt date**	**: XX**
Specimen	**: Peripheral Blood**	**Report date**	**: XX**

Clinical history

Proband, Baby. ZZZ, born of a consanguineous marriage presented with congenital deafness, cerebral palsy, and delayed milestones. No history of fever or infection was reported. Baby Al Hubaishi Malak has been evaluated for pathogenic variations.

Results

Compound heterozygous variants related to the phenotype was identified

List of variants identified related to the phenotype:

Gene	Region	Variant*	Allele Status	Disease	Classification*	Inheritance pattern
PCDH15 (-)	Exon 14	c.1736_1737insAA **(p.Tyr579Ter)**	Heterozygous	Deafness,autosoal recessive 23 **(OMIM#609533)**	**Likely Pathogenic**	Autosomal Recessive
	Exon 6	c.536T>A **(p.Ile179Lys)**		Usher syndrome, type 1F **(OMIM#602083)** Usher syndrome, type 1D/F digenic **(OMIM#601067)**	**Uncertain significance**	

*Genetic test results are 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.

Interpretation

Compound heterozygous variants were identified in *PCDH15* gene

Variant 1:*PCDH15*: c.1736_1737insAA

Variant summary: A heterozygous two base pair insertion in exon 14 of the *PCDH15* gene (chr10:g.54153148insTT, NM_001384140.1, Depth: 105x) that results in the premature truncation of the protein at codon 579 (p. Tyr579Ter) was detected. This variant is a frameshift variant which occurs in an exon of *PCDH15* 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 and Literature evidence: A stop gained variant in the same amino acid position (p.Tyr579Ter) has been previously classified as pathogenic in ClinVar database for autosomal recessive non syndromic hearing loss 23 [3]. Another stop gained variant in the same aminoacid position (c.1737C>G; p.Tyr579Ter) has been previously reported in patients affected with Usher syndrome in compound heterozygous state [4].

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

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

This variant has been identified in the sample of her father, Mr. XXX in heterozygous state

Variant 2:*PCDH15*: c.536T>A

Variant summary: A heterozygous missense variation in exon 6 of the *PCDH15* gene (chr10:g.54346423A>T, NM_001384140.1, Depth: 75x) that results in the amino acid substitution of Lysine for Isoleucine at codon 179 (p.Ile179Lys) was detected.

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

Clinical evidence: A missense variant in the same amino acid position (p. Ile179Thr) has been previously classified as variant of uncertain significance in ClinVar database [5].

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

Variant classification: Based on the evidence, this variant is classified as variant of uncertain significance. **In this view, clinical correlation is 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.**

This variant has been identified in the sample of her mother, Mrs. YYY in heterozygous state.

OMIM phenotype: Deafness, autosomal recessive 23 (OMIM#609533) is caused by homozygous mutation in the *PCDH15* gene (OMIM*605514). Usher syndrome, type 1F (OMIM#602083) and Usher syndrome, type 1D/F digenic (OMIM#601067) are caused by homozygous or compound heterozygous mutation in the *PCDH15* gene (OMIM*605514). Usher syndrome type I is an autosomal recessive condition characterized by profound congenital hearing impairment with unintelligible speech, early retinitis pigmentosa (usually evident within the first decade), and constant vestibular dysfunction. Type I is distinguished from type II (276901) on the basis of severity of hearing loss and the extent of vestibular involvement. Type I patients are profoundly deaf, whereas type II patients are 'hard of hearing.' Vestibular function is defective in type I patients, whereas type II patients have normal vestibular function. Patients with type III (USH3; 276902) have progressive hearing loss. Usher syndrome constitutes a group of autosomal recessive disorders characterized by progressive pigmentary retinopathy and sensorineural hearing loss. Phenotypic distinctions are based on auditory and vestibular differences. Persons with forms of Usher syndrome type I (USH1) have congenital severe to profound hearing loss and vestibular dysfunction. These diseases follow autosomal recessive pattern of inheritance [2].

The variants are reported to be in compound heterozygous state. These compound heterozygous variants are found to be in trans status by parental segregation analysis.

Recommendations

- Alternative test is strongly recommended to rule out the deletion/duplication.
- Genetic counselling is recommended.

Methodology

DNA extracted from the blood was used to perform whole exome using whole exome capture kit. The targeted libraries were sequenced to a targeted depth of 80 to 100X using Illumina 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 (3.1.2,2.1.1), 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

Total reads generated	8.11Gb
Data ≥ Q30	96.99%

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.
- Incidental or secondary findings that meet the ACMG guidelines can be given upon request [6]

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.
3. https://www.ncbi.nlm.nih.gov/clinvar/variation/VCV002677548.1
4. Jaijo T, et al. Mutation screening of the PCDH15 gene in Spanish patients with Usher syndrome type I. Mol Vis. 2012;18:1719-26. Epub 2012 Jun 23. PMID: 22815625; PMCID: PMC3398493.
5. https://www.ncbi.nlm.nih.gov/clinvar/variation/VCV000957413.6
6. 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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