Sample ID:	**11009458745**
Sample Type:	Whole Blood
Patient's Name:	***Mr. ABCDEFGHIJKLMPQ***
Gender/Age:	M/22 Days

Data uploaded on:	DD/MM/YYYY	**Report Generated on:**	DD/MM/YYYY

CLINICAL INDICATIONS: Deafness

RESULTS:

List of clinically significant mutations (CSM)

GENE	VARIANT	ZYGOSITY	DISEASE	INHERITANCE	INTERPRETATION
ASS1 (+) **(NM_054012.4)**	Exon 14 c.1168G>A (p.Gly390Arg)	Heterozygous	**CITRULLINEMIA (OMIM#215700)**	Autosomal Recessive	Uncertain Significance
GJB2 (NM_004004.6)	Exon 2 c.35delG (p.Gly12fs)	Homozygous	**DEAFNESS, AUTOSOMAL RECESSIVE 1A (OMIM#220290)**	Autosomal Recessive	**PATHOGENIC**

Genetic test results are reported based on the recommendations of American College of Medical Genetics.
****Sanger validation is recommended for above listed variants.***
*****The significance/classification of the variant(s) may change based on genetic testing in the parents and other family members.***
******Data revaluation performed upon the up gradation of databases used and results may vary in accordance.***

INTERPRETATION:

Variant - 1 Description:

ASS1 (+),c.1168G>A (p.Gly390Arg)

A heterozygous missense variation in exon 14 of the ASS1 gene (chr9:g.130499545G>A; Depth: 206x) that results in the amino acid substitution of Arginine for Glycine at codon 390 (p.Gly390Arg; NM_054012.4) was detected (Table). The observed variation has previously been reported in patients affected with citrullinemia [PMID-30285816]. This variant has not been reported in the 1000 genomes database and has a minor allele frequency of 0.03% in the gnomAD. The in silico predictions# of the variant are probably damaging by SIFT. The reference codon is conserved across species.

Variant - 2 Description:

GJB2;c.35delG(p.Gly12fs)

A homozygous frame shift variant in exon 2 of the GJB2 gene (chr13:g.20189546AC>A; Depth: 69x) that results in a 1 base pair deletion at codon12 (p.Gly12fs; NM_004004.6) was detected. This variant has not been reported in the gnomAD and 1000 genomes database. The reference codon is conserved across species. [PMID:23900770].

RECOMMENDATIONS:

- Genetic counseling is advised for interpretation on the consequences of the variant(s).
- Sanger validation is recommended to ascertain the false calls (highly recommended for low sequencing depth variants).
- Variant segregation can be analyzed by assaying for the specific mutation detected in all first-degree relatives. This may be helpful for individualized monitoring and surveillance recommendations for relatives as well as for future pre-implantation/prenatal applications, if desired.
- If results obtained do not match the clinical findings, additional testing should be considered as per referring clinician's recommendation.

METHODOLOGY:

The Genes2Me Newborn Screening NGS panel was used for sequencing that screens for 335 clinically relevant genes (coding regions of the genome) for 340 diseases associated with genetic mutations in new born babies. It covers all major mutations like SNV and Indels adding up to a approx. target size of 1.5 Mb with hybridization-based target capture technique.

After raw data generation, we follow the GATK best practices framework for identification of variants in the sample, starting with raw data quality check using the FastQC followed by BWA read aligner for mapping/aligning to human reference genome GRCh38. After the alignment, GATK HaplotypeCaller algorithm was used for variant calling. Annotation of the variants was performed using open-source available software SnpEff. Further, clinically relevant mutations were annotated using published variants in literature and set of diseases databases – ClinVar, Franklin, OMIM, and HPO. The gnomAD, dbSNP and 1000 Genome databases were used for annotation of variants for their minor allele frequency. The dbNSFP database was used for annotation and functional prediction of all potential non-synonymous variants.

VARIANT CLASSIFICATION AS PER "ACMG GUIDELINES":

Variant is a permanent change in the sequence of DNA that makes up a gene that could be pathogenic – causing a disease or benign – not causing a disease.

1. **Pathogenic Variant**
   The variant is responsible for causing disease with ample scientific research to support the disease – gene – variant association. These variants are often referred to as mutations.

2. **Likely Pathogenic Variant**
   The variant is very likely responsible to contribute to the development of disease with insufficient scientific evidences to prove the association. Additional evidences and testing is expected to confirm this assertion of pathogenicity.
3. **Variant of Uncertain Significance**
   The variant whose impact is difficult to classify based on current available scientific evidences. Further testing of the patient or family members is recommended.

LIMITATIONS:

- Genetic testing is an important part of the diagnostic process however it may not always give a definitive answer. In some cases, a genetic variant may be missed due to the limitations in existing medical knowledge and testing technology. Accurate interpretation of test results is dependent on the availability of biological & medical information (clinical history) of the family, failing to this may leads to incorrect result interpretation and diagnosis.
- Test results are interpreted in the context of clinical findings, available scientific evidences, family history and other laboratory data. The variation(s) which is/are potentially relevant – significant related to the patient's provided medical history is/are reported.
- Genetic testing is highly accurate but rarely inaccurate results may occur for various reasons like mislabeling of samples, inaccurate clinical/medical family history, rare technical errors or unusual circumstances such as bone marrow transplantation, blood transfusion or the presence of change(s) in such a small percentage of cells that may not be detectable (mosaicism).

DISCLAIMER:

- The interpretation of variants in this report is performed to the best knowledge of the scientific & medical information available at the time of reporting. The classification of variants is based on ACMG (American College of Medical Guidelines) guidelines.
- As of the inherent technological limitations of the sequencing assay, some of the coding regions can't be properly sequenced, so, variations in these regions may not be identified & interpreted. It is possible that variants not identified by the assay may be associated with the provided phenotypes of the patient.
- It is possible due to insufficient phenotypic information, a gene – variant may be present in data but not selected for analysis and interpretation.
- The mutations have not been confirmed by Sanger sequencing.
- Genes2Me clarify that the generated report(s) doesn't provide any kind of diagnosis or opinion or recommendation for any disease and its cure in any manner. It is therefore recommended that the patient and/or the guardian(s) of the patient must take the consultation of the clinician or a certified physician or doctor for further course of action.
- If the provided material quality and/or quantity not up to the desired level, so, in that case, test(s) result(s) may be misleading or even wrong, therefore, Genes2Me hereby disclaims all liability arising in this connection with the test(s) and report(s).
- The analysis pipeline is developed in-house and the performance characteristics of this analysis are determined by Genes2Me only.
- This test result should be used as a reference by the healthcare provider for diagnosis and development of treatment plan.

REFERENCES:

1. Richards S *et al*., ACMG Laboratory Quality Assurance Committee – 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. Genet Med. 2015 May;17(5):405-24.
2. Kalia SS *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. 2017 Feb;19(2):249-255.
3. Andrews S, FastQC: A Quality Control Tool for High Throughput Sequence Data, 2010, (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/).
4. Bolger AM *et al*., Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics (Oxford, England) (2014) Vol. 30,15: 2114-20.
5. Li H. *et al.*, Fast and accurate long-read alignment with Burrows-Wheeler transform. Bioinformatics (2010), 26(5):589-95.
6. Quinlan AR *et al.*, BEDTools: a flexible suite of utilities for comparing genomic features. Bioinformatics (Oxford, England) (2010) Vol. 26,6: 841-2.

7. McKenna A *et al*., The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome research (2010) Vol. 20,9: 1297-303.
8. Cingolani P *et al*., A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w1118; iso-2; iso-3. Fly (2012) Vol. 6,2: 80-92.
9. Clarke L *et al*. The international Genome sample resource (IGSR): A worldwide collection of genome variation incorporating the 1000 Genomes Project data. Nucleic acids research (2017) Vol. 45,D1: D854-D859.
10. Karczewski KJ *et al*., The mutational constraint spectrum quantified from variation in 141,456 humans. Nature (2020) Vol. 581,7809: 434-443.
11. GenomeAsia100K Consortium. The GenomeAsia 100K Project enables genetic discoveries across Asia. Nature (2019) 576, 106–111.
12. Landrum MJ *et al*., ClinVar: improving access to variant interpretations and supporting evidence. Nucleic acids research (2018) Vol. 46,D1: D1062-D1067.
13. McKusick VA, Mendelian Inheritance in Man. A Catalog of Human Genes and Genetic Disorders. Baltimore: Johns Hopkins University Press (12th edition), 1998.
14. Köhler S *et al*., The Human Phenotype Ontology in 2021. Nucleic acids research (2021) Vol. 49,D1: D1207-D1217.
15. Sherry ST *et al.*, dbSNP—Database for Single Nucleotide Polymorphisms and Other Classes of Minor Genetic Variation. Genome Res. (1999), 9, 677–679.
16. Liu, X *et al*., dbNSFP v4: a comprehensive database of transcript-specific functional predictions and annotations for human nonsynonymous and splice-site SNVs. Genome Med (2020) Vol. 12, 103.
17. Franklin Link:- https://franklin.genoox.com/clinical-db/home

*

END OF REPORT