Client: Example Client ABC123
123 Test Drive
Salt Lake City, UT 84108
UNITED STATES

Physician: Doctor, Example

Patient: Patient, Example

DOB	5/22/2023
Gender:	Male
Patient Identifiers:	01234567890ABCD, 012345
Visit Number (FIN):	01234567890ABCD
Collection Date:	00/00/0000 00:00

Connexin 26 (GJB2) Sequencing and Deletion/Duplication

ARUP test code 3004720

CX26 Specimen: Whole Blood

CX26 Interp: Positive

RESULT
Two pathogenic variants were detected in the GJB2 gene.

PATHOGENIC VARIANT
Gene: GJB2 (NM_004004.6)
Nucleic Acid Change: c.299_300del; Heterozygous
Amino Acid Alteration: p.His100ArgfsTer14
Inheritance: Autosomal recessive

PATHOGENIC-MILD VARIANT
Gene: GJB2 (NM_004004.6)
Nucleic Acid Change: c.109G>A; Heterozygous
Amino Acid Alteration: p.Val37Ile
Inheritance: Autosomal recessive

INTERPRETATION
One pathogenic and one mildly pathogenic variant, c.299_300del; p.His100ArgfsTer14, and c.109G>A; p.Val37Ile, were detected in the GJB2 (Connexin 26) gene by massively parallel sequencing. Pathogenic variants in GJB2 are commonly associated with autosomal recessive deafness-1A (DFNB1A, MIM: 220290; OMIM(R)). Individuals who carry the identified pathogenic GJB2 variants on opposite chromosomes may have mild to moderate nonsyndromic hearing loss. Based on the sequencing reads, we were able to determine that these two pathogenic variants are on opposite chromosomes.

Please refer to the background information included in this report for the methodology and limitations of this test.

Evidence for variant classification(s): The GJB2 c.299_300del; p.His100ArgfsTer14 variant (rs111033204) is reported in the literature in multiple individuals affected with nonsyndromic hearing loss (Abe 2000, Chen 2016, Huang 2013, Wang 2002). Several affected individuals with this variant were also observed to carry a second pathogenic variant (Abe 2000, Huang 2013, Wang 2002), including two siblings confirmed to carry another frameshift variant in trans to p.His100ArgfsTer14 (Wang 2002). This variant is found in the East Asian population with an overall allele frequency of 0.09% (18/19950 alleles) in the Genome Aggregation Database (v2.1.1), and it is reported as pathogenic by multiple laboratories in ClinVar (Variation ID: 44736). This variant causes a frameshift by deleting two nucleotides, so it is predicted to result in a truncated protein or mRNA subject to nonsense-mediated decay. Based on available

information, this variant is considered to be pathogenic.

The GJB2 c.109G>A; p.Val37Ile variant (rs72474224) is reported in the literature in multiple individuals affected with hearing loss (Baux 2017, Kecskemeti 2018, Posukh 2019, Putcha 2007, Zhou 2019). However, homozygosity for p.Val37Ile has been associated with normal hearing (Tang 2006) as well as with slight, mild or moderate hearing loss, primarily in individuals of Asian background (Bason 2002, Dahl 2006, Kim 2015, Kim 2013, Pollak 2007, Rabionet 2000). The p.Val37Ile variant is reported as pathogenic by several laboratories in ClinVar (Variation ID: 17023) and has been shown to disrupt protein function using a Xenopus oocyte-based gap junction formation assay (Bruzzone 2003) and a gap junction permeability assay in HEK293 cells (Kim 2015). This variant is found predominantly in the East Asian population with an allele frequency of 8.3% (1665/19952 alleles, including 96 homozygotes) in the Genome Aggregation Database (v2.1.1). Additionally, other variants at this codon (c.110T>C; p.Val37Ala and c.109G>C; p.Val37Leu) have been reported in individuals with sensorineural hearing loss (Azaiez 2004, Putcha 2007). Based on available information, this variant is considered to be mildly pathogenic.

RECOMMENDATIONS
Medical management should rely on clinical findings and family history. Family members should be offered targeted testing for the identified pathogenic variants (Familial Targeted Sequencing, ARUP test code 3005867). This individual's future reproductive partner should be offered screening for GJB2 and GJB6 pathogenic variants.

COMMENTS
Likely benign and benign variants are not reported.
Variants in the following region(s) may not be detected by NGS with sufficient confidence in this sample due to technical limitations: None

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This result has been reviewed and approved by [redacted]

BACKGROUND INFORMATION: Connexin 26 (GJB2) Sequencing and Deletion/Duplication

CHARACTERISTICS: Biallelic autosomal recessive GJB2 variants are associated with autosomal recessive deafness type 1A (DFNB1A), characterized by mild to profound nonsyndromic hearing loss (NSHL) that is typically nonprogressive. A pathogenic GJB2 variant on the opposite chromosome from a large GJB6 gene deletion, involving GJB2 cis-regulatory elements and loss of expression of GJB2, is also causative for NSHL. Autosomal dominant GJB2 sequence variants are causative for a variety of phenotypes including autosomal dominant deafness type 3A (DFNA3A) and syndromic forms of hearing loss which may include keratosis or ichthyosis.

EPIDEMIOLOGY: Approximately 1:6500

CAUSE: Pathogenic germline variants in GJB2

INHERITANCE: Autosomal recessive or dominant depending on specific GJB2 variant

CLINICAL SENSITIVITY: Greater than 99 percent for GJB2-associated hearing loss (DFNB1A)

GENE TESTED: GJB2 (NM_004004).

METHODOLOGY: Probe hybridization-based capture of all coding exons, exon-intron junctions, and selected noncoding variants of the GJB2 gene, followed by massively parallel sequencing. Sanger sequencing was performed as necessary to fill in regions of low coverage and to confirm reported variants that do not meet acceptable quality metrics. A proprietary bioinformatic algorithm was used to detect large (single exon-level or larger) deletions or duplications in the GJB2 gene. Large deletions/duplications were confirmed using an orthogonal exon-level microarray. Human genome build 19 (Hg 19) was used for data analysis.

ANALYTICAL SENSITIVITY/SPECIFICITY: The analytical sensitivity

is approximately 99 percent for single nucleotide variants (SNVs) and greater than 93 percent for insertions/duplications/deletions (indels) from 1-10 base pairs in size. Indels greater than 10 base pairs may be detected, but the analytical sensitivity may be reduced. Deletions and duplications of the full GJB2 coding exon are detected with 97 percent and 83 percent sensitivity, respectively. Specificity is greater than 99.9 percent for all variant classes.

LIMITATIONS: A negative result does not exclude a heritable form of hearing loss. This test only detects variants within the coding regions, intron-exon boundaries, and selected noncoding variants of the GJB2 gene. Deletions/duplications/insertions of any size may not be detected by massively parallel sequencing. Regulatory region variants and deep intronic variants will not be identified. Diagnostic errors can occur due to rare sequence variations. In some cases, variants may not be identified due to technical limitations caused by the presence of pseudogenes, repetitive, or homologous regions. This test is not intended to detect low-level mosaic or somatic variants, gene conversion events, complex inversions, translocations, mitochondrial DNA (mtDNA) variants, or repeat expansions. Interpretation of this test result may be impacted if this patient has had an allogeneic stem cell transplantation. Noncoding transcripts were not analyzed.

This test was developed and its performance characteristics determined by ARUP Laboratories. It has not been cleared or approved by the U.S. Food and Drug Administration. This test was performed in a CLIA-certified laboratory and is intended for clinical purposes.

Counseling and informed consent are recommended for genetic testing. Consent forms are available online.

VERIFIED/REPORTED DATES

Procedure	Accession	Collected	Received	Verified/Reported
CX26 Specimen	24-304-149875	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00
CX26 Interp	24-304-149875	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00

END OF CHART

H=High, L=Low, *=Abnormal, C=Critical