MORL OtoSCOPE® Reports

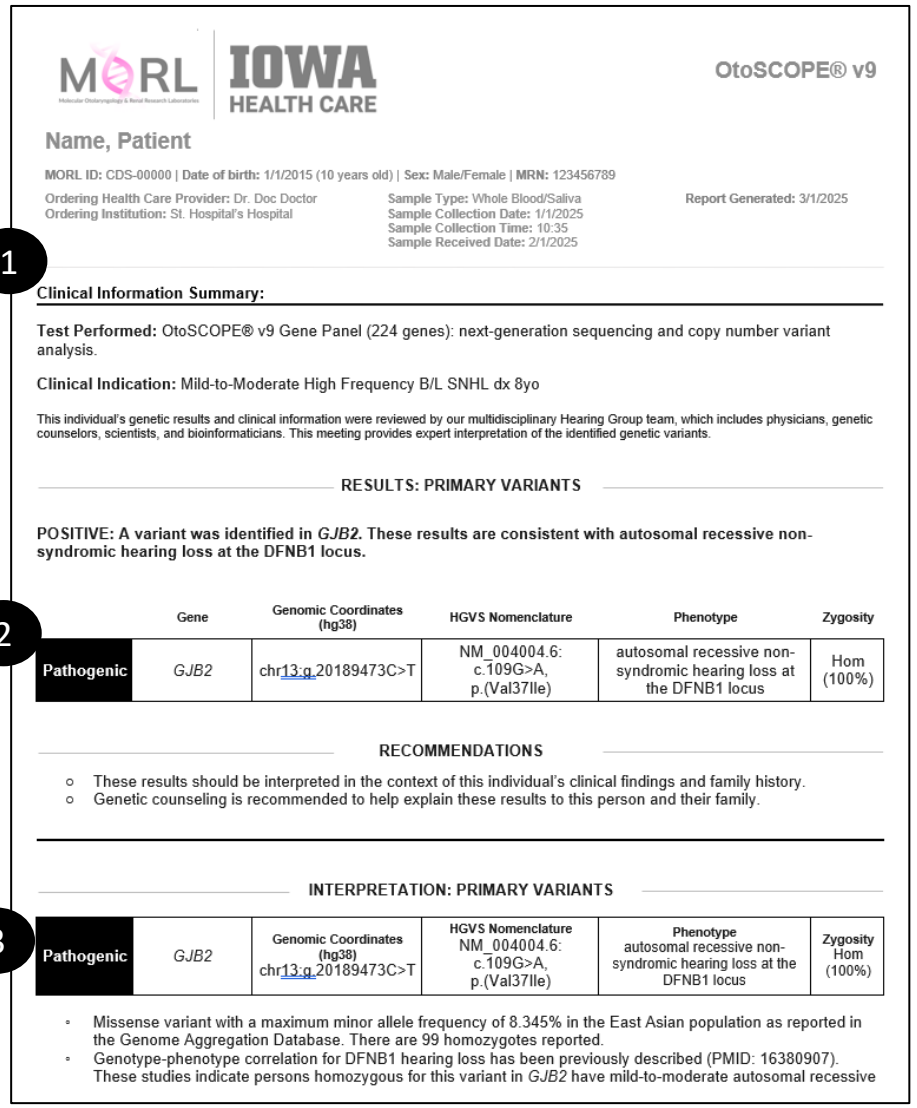
A Guide to MORL's Enhanced Reports

Introducing MORL's Enhanced Genetic Reports!
Launching for samples received on and after April 1, 2025

Each section, numbered for your convenience, is detailed in the following pages.

Test improvements:

- Transitioning to GRCh38 Genome Build
- Expanded Coverage of intronic regions



MORL | IOWA HEALTH CARE — OtoSCOPE® v9

Name, Patient

MORL ID: CDS-00000 | Date of birth: 1/1/2015 (10 years old) | Sex: Male/Female | MRN: 123456789

Ordering Health Care Provider: Dr. Doc Doctor
Ordering Institution: St. Hospital's Hospital

Sample Type: Whole Blood/Saliva
Sample Collection Date: 1/1/2025
Sample Collection Time: 10:35
Sample Received Date: 2/1/2025

Report Generated: 3/1/2025

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Clinical Information Summary:

Test Performed: OtoSCOPE® v9 Gene Panel (224 genes): next-generation sequencing and copy number variant analysis.

Clinical Indication: Mild-to-Moderate High Frequency B/L SNHL dx 8yo

This individual's genetic results and clinical information were reviewed by our multidisciplinary Hearing Group team, which includes physicians, genetic counselors, scientists, and bioinformaticians. This meeting provides expert interpretation of the identified genetic variants.

RESULTS: PRIMARY VARIANTS

POSITIVE: A variant was identified in *GJB2*. These results are consistent with autosomal recessive non-syndromic hearing loss at the DFNB1 locus.

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	Gene	Genomic Coordinates (hg38)	HGVS Nomenclature	Phenotype	Zygosity
Pathogenic	*GJB2*	chr13:g.20189473C>T	NM_004004.6: c.109G>A, p.(Val37Ile)	autosomal recessive non-syndromic hearing loss at the DFNB1 locus	Hom (100%)

RECOMMENDATIONS

- These results should be interpreted in the context of this individual's clinical findings and family history.
- Genetic counseling is recommended to help explain these results to this person and their family.

INTERPRETATION: PRIMARY VARIANTS

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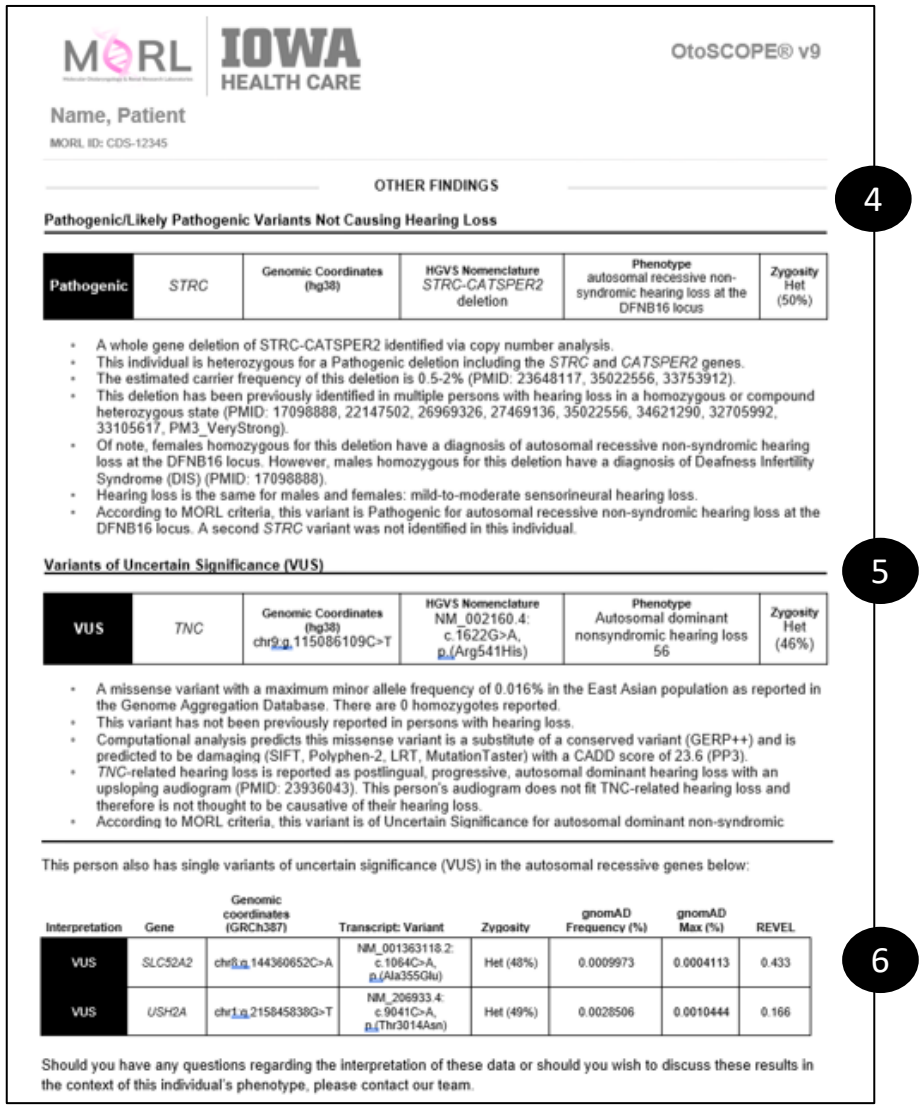
		Genomic Coordinates (hg38)	HGVS Nomenclature	Phenotype	Zygosity
Pathogenic	*GJB2*	chr13:g.20189473C>T	NM_004004.6: c.109G>A, p.(Val37Ile)	autosomal recessive non-syndromic hearing loss at the DFNB1 locus	Hom (100%)

- Missense variant with a maximum minor allele frequency of 8.345% in the East Asian population as reported in the Genome Aggregation Database. There are 99 homozygotes reported.
- Genotype-phenotype correlation for DFNB1 hearing loss has been previously described (PMID: 16380907). These studies indicate persons homozygous for this variant in *GJB2* have mild-to-moderate autosomal recessive



MORL | IOWA HEALTH CARE — OtoSCOPE® v9

Name, Patient

MORL ID: CDS-12345

OTHER FINDINGS

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Pathogenic/Likely Pathogenic Variants Not Causing Hearing Loss

		Genomic Coordinates (hg38)	HGVS Nomenclature	Phenotype	Zygosity
Pathogenic	*STRC*		*STRC-CATSPER2* deletion	autosomal recessive non-syndromic hearing loss at the DFNB16 locus	Het (50%)

- A whole gene deletion of STRC-CATSPER2 identified via copy number analysis.
- This individual is heterozygous for a Pathogenic deletion including the *STRC* and *CATSPER2* genes.
- The estimated carrier frequency of this deletion is 0.5-2% (PMID: 23648117, 35022556, 33753912).
- This deletion has been previously identified in multiple persons with hearing loss in a homozygous or compound heterozygous state (PMID: 17098888, 22147502, 26969326, 27469136, 35022556, 34621290, 32705992, 33105617, PM3_VeryStrong).
- Of note, females homozygous for this deletion have a diagnosis of autosomal recessive non-syndromic hearing loss at the DFNB16 locus. However, males homozygous for this deletion have a diagnosis of Deafness Infertility Syndrome (DIS) (PMID: 17098888).
- Hearing loss is the same for males and females: mild-to-moderate sensorineural hearing loss.
- According to MORL criteria, this variant is Pathogenic for autosomal recessive non-syndromic hearing loss at the DFNB16 locus. A second *STRC* variant was not identified in this individual.

Variants of Uncertain Significance (VUS)

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		Genomic Coordinates (hg38)	HGVS Nomenclature	Phenotype	Zygosity
VUS	*TNC*	chr9:g.115086109C>T	NM_002160.4: c.1622G>A, p.(Arg541His)	Autosomal dominant nonsyndromic hearing loss 56	Het (46%)

- A missense variant with a maximum minor allele frequency of 0.016% in the East Asian population as reported in the Genome Aggregation Database. There are 0 homozygotes reported.
- This variant has not been previously reported in persons with hearing loss.
- Computational analysis predicts this missense variant is a substitute of a conserved variant (GERP++) and is predicted to be damaging (SIFT, Polyphen-2, LRT, MutationTaster) with a CADD score of 23.6 (PP3).
- *TNC*-related hearing loss is reported as postlingual, progressive, autosomal dominant hearing loss with an upsloping audiogram (PMID: 23936043). This person's audiogram does not fit TNC-related hearing loss and therefore is not thought to be causative of their hearing loss.
- According to MORL criteria, this variant is of Uncertain Significance for autosomal dominant non-syndromic

This person also has single variants of uncertain significance (VUS) in the autosomal recessive genes below:

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Interpretation	Gene	Genomic coordinates (GRCh37)	Transcript: Variant	Zygosity	gnomAD Frequency (%)	gnomAD Max (%)	REVEL
VUS	*SLC52A2*	chr8:g.144360652C>A	NM_001363118.2: c.1064C>A, p.(Ala355Glu)	Het (48%)	0.0009973	0.0004113	0.433
VUS	*USH2A*	chr1:g.215845838G>T	NM_206933.4: c.9041C>A, p.(Thr3014Asn)	Het (49%)	0.0028506	0.0010444	0.166

Should you have any questions regarding the interpretation of these data or should you wish to discuss these results in the context of this individual's phenotype, please contact our team.

MORL
Molecular Otolaryngology & Renal Research Laboratories

Name, Patient

MORL ID: CDS-00000

SAMPLE TEST PERFORMANCE

Coverage of Targeted Regions:

- 14598790 sequencing reads (150bp, paired-end) were aligned.
- 98.42% of 1.647 Mbp base pairs targeted were covered at greater than 30X depth of coverage.

MATERIALS AND METHODS

Panel Description

The OtoSCOPE® v9 panel platform includes 224 genes associated with non-syndromic and syndromic hearing loss and deafness.

GENES ANALYZED: *ABHD12, ACTB, ACTG1, ADCY1, ADGRV1, AIFM1, ALMS1, AMMECR1, ANKH, ATP2B2, ATP6V0A4, ATP6V1B1, ATP6V1B2, BCS1L, BDP1, BSND, BTD, CABP2, CACNA1D, CCDC50, CD164, CDC14A, CDH23, CEACAM16, CEP78, CHD7, CHSY1, CIB2, CISD2, CLDN14, CLDN9, CLIC5, CLPP, CLRN1, COCH, COL11A1, COL11A2, COL2A1, COL4A3, COL4A4, COL4A5, COL4A6, COL9A1, COL9A2, COL9A3, CRYM, DCAF17, DCDC2, DIABLO, DIAPH1, DIAPH3, DLX5, DMXL2, DNMT1, DSPP, EDN3, EDNRB, ELMOD3, EPS8, EPS8L2, ERAL1, ESPN, ESRRB, EYA1, EYA4, FDXR, FGF3, FGFR1, FGFR2, FGFR3, FITM2, FOXI1, GAB1, GATA3, GIPC3, GJB2, GJB3, GJB6*[†]*, GPRASP2, GPSM2, GRAP, GREB1L, GRHL2, GRXCR1, GRXCR2, GSDME, HARS2, HGF, HOMER2, HOXA2, HOXB1, HSD17B4, IFNLR1, ILDR1, KARS1, KCNE1, KCNJ10, KCNQ1, KCNQ4, KITLG, KMT2D, LARS2, LHFPL5, LHX3, LMX1A, LOXHD1, LOXL3, LRP2, LRTOMT, MAN2B1, MANBA, MARVELD2, MASP1, MCM2, MET, MGP, MIR96, MITF, MPZL2, MSRB3, MT-CO1, MT-ND1, MT-RNR1, MT-TH, MT-TI, MT-TK, MT-TL1, MT-TS1, MT-TS2, MYH14, MYH9, MYO15A, MYO3A, MYO6, MYO7A, NARS2, NDP, NEFL, NF2, NLRP3, NOG, NR2F1, OPA1, OSBPL2, OTOA, OTOF, OTOG, OTOGL, P2RX2, PAX1, PAX3, PCDH15, PDE1C, PDZD7, PEX1, PEX26, PEX6, PJVK, PLS1, PNPT1, POLR1B, POLR1C, POLR1D, POU3F4, POU4F3, PPIP5K2, PRPS1, PTPRQ, RAI1, RDX, REST, RIPOR2, ROR1, S1PR2, SEMA3E, SERPINB6, SIX1, SIX2, SIX5, SLC17A8, SLC19A2, SLC22A4, SLC26A4, SLC26A5, SLC33A1, SLC44A4, SLC4A11, SLC52A2, SLC52A3, SLITRK6, SMPX, SNAI2, SOX10, SPATA5, SPNS2, STRC, SUCLA2, SYNE4, TBC1D24, TBL1X, TBX1, TCOF1, TECTA, TFAP2A, TIMM8A, TJP2, TMC1, TMEM126A, TMEM132E, TMIE, TMPRSS3, TNC, TPRN, TRIOBP, TRRAP, TSPEAR, TUBB4B, TWNK, USH1C, USH1G, USH2A, WBP2, WFS1, WHRN*

[†]Genomic Regions covered for copy number variant analysis only. Additional genomic regions covered for copy number variant analysis: *CATSPER2, CRYL1, OTOAP1, STRCP1*. For a complete list of OtoSCOPE® v9 genes, phenotypes, OMIM identifiers, and inheritance, visit: https://morl.lab.uiowa.edu/clinical-diagnostic-services/hearing-loss-clinical-division/otoscoper-genetic-hearing-loss-testing.

Terms and abbreviations

- **Variant**: a difference from the reference genomic sequence that was detected in the sample
- **SNV**: single nucleotide variant, such as a nucleotide substitution
- **Indel**: an insertion or deletion of bases in the nucleotide sequence, typically smaller than 100 bases
- **CNV**: copy number variant; a deletion or duplication of sequence larger than an indel
- **HGVS Nomenclature**: description of the variant using Human Genome Variation Society (HGVS) standards, typically with a cDNA (c.) and/or protein (p.) reference sequence
- **Zygosity**: the relation of the variant to the presence of a reference allele at the same position
  - heterozygous (**het**): the variant is present on one of two alleles
  - homozygous (**hom**): the variant is present on both of two alleles
  - hemizygous (**hemi**): the variant is present on a single allele (either a hemizygous sex chromosome, or with a deletion of the other allele on an autosome)
- **gnomAD**: the Genome Aggregation Database is composed of exome and genome sequences from around the world, providing minor allele frequencies across continent-scale populations
- **REVEL score**: Rare Exome Variant Ensemble Learner (REVEL) is an ensemble method for predicting the pathogenicity of missense variants based on a combination of scores from 13 individual pathogenicity and conservation predictors. The REVEL score for an individual missense variant can range from 0 to 1, with higher scores reflecting greater likelihood that the variant is disease-causing.
- **Interpretation:** Variant interpretation reflects MORL expert curation (Azaiez H, Booth KT, et al. 2018). It is based on decades of experience in the genetics of hearing loss, data extracted from literature review, genotype-phenotype studies, ClinVar and HGMD, and follows the American College of Medical Genetics and Association of Medical Pathology guidelines and nomenclature for Hearing Loss (Oza et al., 2018).
  - Pathogenic (**P**): a disease-causing variant
  - Likely Pathogenic (**LP**): a variant likely to be disease causing, but more evidence is needed to prove this conclusively
  - Variant of Uncertain Significance (**VUS**): a variant that has an uncertain or unknown impact on disease, either because of a lack of evidence or conflicting evidence.
  - Likely Benign (**LB**): a variant not likely to be disease causing but more evidence is needed to prove this conclusively.

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MORL IOWA HEALTH CARE

OtoSCOPE® v9

Name, Patient

MORL ID: CDS-00000

- Benign (**B**): a variant that does not cause disease

Detailed Description of OtoSCOPE® Testing

OTOSCOPE® TARGETED GENOMIC ENRICHMENT AND SEQUENCING

The OtoSCOPE® v9 (224 genes) platform uses solution-phase targeted genomic enrichment to isolate and enrich all exons and their flanking intronic sequence, and intronic deafness-causing variants. Also included are some intronic, intergenic, and pseudogene sequences to improve our ability to detect copy number variations. Sequencing by reversible chain-termination is performed using Illumina systems with post-capture barcoded multiplexing and 150 bp paired-end reads.

BIOINFORMATIC ANALYSIS

Raw sequence data are demultiplexed and converted to FASTQ files with Illumina bcl2fastq v2. FASTQ files are securely transferred to Franklin by Gencox for analysis. The Franklin system is HIPAA compliant and a Business Associate Agreement for data handling is in place. Reads are aligned to reference genome GRCh38. SNV and indels are called with multiple variant callers; CNV are called with a read depth model based on well-studied validation batches.

COVERAGE AND SENSITIVITY

Coverage refers to the percentage of the defined target region where the read depth is at least 30X. To meet our quality control (QC) standards, this test must achieve a coverage threshold of 97.7%. However, due to genomic regions with high homology, certain areas in this test cannot be confidently covered in any sample. If these regions are excluded from the coverage calculations, the threshold increases to 99%. For regions with 30X or higher coverage, the analytical sensitivity (variant detection) is greater than 99%.

VARIANT FILTERING

Variants are filtered for review based on confidence and quality of variant call, population frequency, impact on gene (coding region and +/-20 intronic bases), prior publication or public case data, and prior internal or external variant interpretation. Variants with minor allele frequencies (MAFs) > 1% in the Genome Aggregation Database (gnomAD) are filtered out with exceptions made for previously identified pathogenic variants, which are retained for consideration. All retained variants classified as VUS, LP, or P are displayed in this report and are discussed at the multidisciplinary hearing meeting (Hearing Group) by a panel of experts. Variants classified as LB or B are not included in the report but are available upon request.

VARIANT INTERPRETATION

Variant interpretation is complex and relies on all available clinical, phenotypic, and genetic information. There is no single method, tool, or filter that reliably determines pathogenicity and so expert analysis is required. The goal of our multidisciplinary Hearing Group is to integrate clinical information with results from OtoSCOPE® to determine the most likely genetic cause of deafness, if any. All variants included in this report are discussed. The Hearing Group meeting comprises experts in the genetics and molecular biology of hearing and deafness and includes physicians, geneticists, scientists, genetic counselors, and bioinformaticians who discuss each variant in the context of clinical information provided and provide expert interpretation of genetic variants. Exclusion of variants as causative is a complex process. Possible justifications for exclusion of a variant may include any of the following but are not limited to: 1) Causality of the identified variant(s) is not consistent with the reported family history; 2) Causality of the identified variant(s) is not consistent with the clinical description and/or phenotypic information provided to MORL; 3) Causality of the identified variant(s) is unlikely based on *in silico* prediction tools; 4) Allele frequency is too high given the prevalence of the associated phenotype or condition.

NGS CONFIRMATORY TESTING, SAMPLE INTEGRITY, AND FAMILIAL TESTING

We have experimentally verified that variants with QD>10 do not require Sanger sequencing validation (PMID: 23804846). In cases where the QD is between 5 and 10 and/or the zygosity status is indeterminate for a Likely Pathogenic/Pathogenic variant, confirmation using Sanger sequencing is performed. Quality control testing to detect specimen mislabeling or sample contamination (sample integrity check) is performed for all samples. A genotyping panel of 12 SNPs used to infer ethnicity (PMID: 24508742, 26355664) is obtained from the OtoSCOPE® Panel and verified against genotypes obtained by allelic discrimination PCR from a separate DNA extraction. Familial testing is available for variants detected with OtoSCOPE® Panel. Please see the MORL testing menu for details (Testing Menu | Molecular Otolaryngology and Renal Research Laboratories - The University of Iowa).

LIMITATIONS OF OTOSCOPE®

OtoSCOPE® is designed to evaluate the exonic and flanking intronic sequence of genes involved in non-syndromic hearing loss and select types of syndromic hearing loss. Deep intronic or regulatory variants of the genes included in OtoSCOPE® v9 (224 genes) are not included in this assay unless they were known to be Pathogenic at time of test design, and some variant types such as genomic rearrangements are not detectable with the test methods. In most cases, it is not possible to determine phase of multiple variants in a single individual. Absence of a plausible explanation for a person's hearing loss by the panel does not exclude a genetic basis for this person's hearing loss. For example, it is possible that the genomic region was not captured on this test version (e.g., a novel gene). OtoSCOPE® testing is performed on DNA extracted from peripheral blood, buccal swab, or saliva, and

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MORL IOWA HEALTH CARE

OtoSCOPE® v9

Name, Patient

MORL ID: CDS-00000

detected heteroplasmy is reflective of the provided sample type only. Tissue-specific heteroplasmy (i.e., inner ear) or somatic changes are not detected by this test.

REGIONS NOT COVERED ON OtoSCOPE® v9

Genomic regions within the 224 genes of OtoSCOPE® v9 that are not covered include: *PTPRQ*: NM_001145026.2: exons 4-5. Genes with highly homologous pseudogenes or paralogs may have lower coverage and detection of variants with NGS panels. Impacted genes/regions in OtoSCOPE® v9 include: *GRAP*: NM-006613.4: exons 1-3; *KCNE1*: NM_000219.6: exon 4; *OTOA*: NM_114672.4: exons 23-26, 28; *STRC*: NM_153700.2: exons 1-3, 5-13, 15, 29). For more details on common areas of low coverage, please see our website: https://morl.lab.uiowa.edu/clinical-diagnostic-services/hearing-loss-clinical-division/otoscoper-genetic-hearing-loss-testing.

SECONDARY AND INCIDENTAL FINDINGS

OtoSCOPE® v9 gene panel may identify genetic findings that are not directly related to the cause of hearing loss or deafness in the individual being tested. These findings, referred to as secondary and incidental findings, may be reviewed and reported as part of this test as they may be of medical value to this person and/or their family members. The MORL follows guidelines for secondary findings from the American College of Medical Genetics and Genomics (ACMG) and has stringent criteria for reporting non-hearing loss related phenotypes. Markers across the X and Y chromosomes are analyzed for quality control purposes and may detect deviations from the expected sex chromosome complement. Such deviations may be included in the report in accordance with MORL policy. Please see our website for more details on our Secondary and Incidental Findings Policy: https://morl.lab.uiowa.edu/clinical-diagnostic-services/hearing-loss-clinical-division/otoscoper-genetic-hearing-loss-testing.

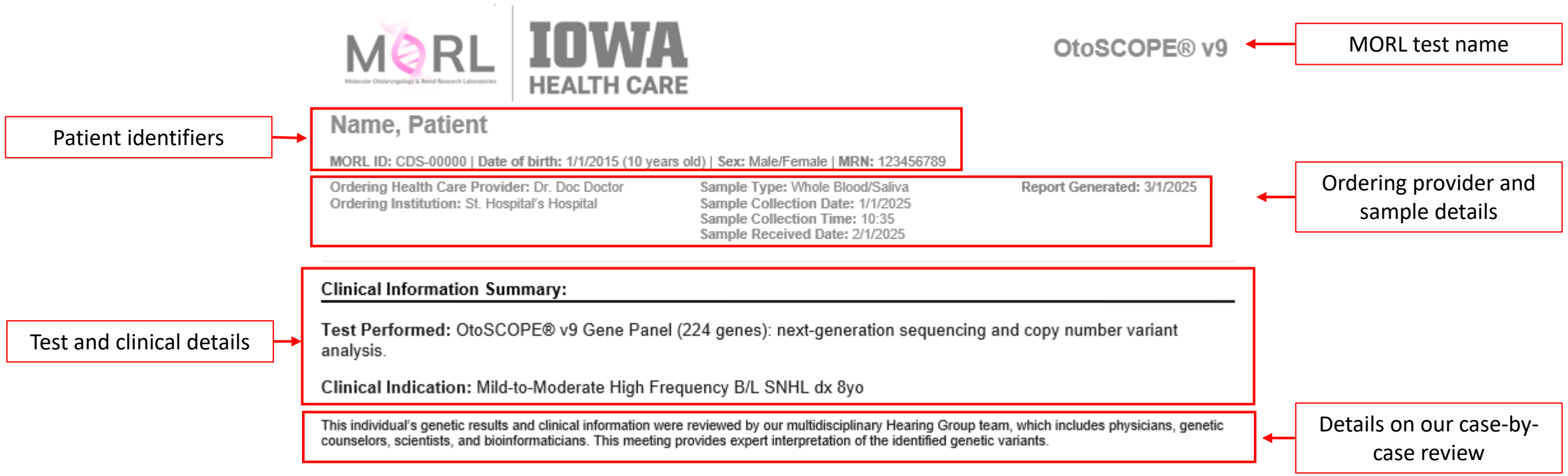
COUNSELING RECOMMENDATIONS

Correlation of variants identified through OtoSCOPE® testing in the context of an individual's clinical phenotype is recommended. Segregation analysis to determine inheritance and phase of variants is recommended. Genetic counseling may be beneficial for an individual and their family to understand the significance of identified variants.

For further details please refer to:

- Azaiez H, Booth KT, Ephraim SS, et al. Genomic Landscape and Mutational Signatures of Deafness-Associated Genes. *Am J Hum Genet*. 2018;103(4):484-497. doi:10.1016/j.ajhg 2018.08.006. PMID: 30245029.
- Chen S, Francioli LC, Goodrich JK, et al. A genomic mutational constraint map using variation in 76,156 human genomes [published correction appears in Nature. 2024 Feb;626(7997):E1. doi: 10.1038/s41586-024-07050-7.]. *Nature*. 2024;625(7993):92-100. doi:10.1038/s41586-023-06045-0. PMID: 38057664.
- Ioannidis NM, Rothstein JH, Pejaver V, et al. REVEL: An Ensemble Method for Predicting the Pathogenicity of Rare Missense Variants. *Am J Hum Genet*. 2016;99(4):877-885. doi:10.1016/j.ajhg 2016.08.016. PMID: 27666373.
- Oza AM, DiStefano MT, Hemphill SE, et al. Expert specification of the ACMG/AMP variant interpretation guidelines for genetic hearing loss. *Hum Mutat*. 2018;39(11):1593-1613. doi:10.1002/humu.23630. PMID: 30311386.
- Shearer AE, Black-Ziegelbein EA, Hildebrand MS, et al. Advancing genetic testing for deafness with genomic technology. *J Med Genet*. 2013;50(9):627-634. doi:10.1136/jmedgenet-2013-101749. PMID: 23804846.
- Shearer AE, Kolbe DL, Azaiez H, et al. Copy number variants are a common cause of non-syndromic hearing loss. *Genome Med*. 2014;6(5):37. Published 2014 May 22. doi:10.1186/gm554. PMID: 24963352.
- Shearer AE, Smith RJ. Massively Parallel Sequencing for Genetic Diagnosis of Hearing Loss: The New Standard of Care. *Otolaryngol Head Neck Surg*. 2015;153(2):175-182. doi:10.1177/0194599815591156. PMID: 26084827.
  Sloan-Heggen CM, Bierer AO, Shearer AE, et al. Comprehensive genetic testing in the clinical evaluation of 1119 patients with hearing loss. *Hum Genet*. 2016;135(4):441-450. doi:10.1007/s00439-016-1648-8. PMID: 26969326.

1 Report Introduction



2 Results Summary and Recommendations

Variants that are causative (or potentially causative) of hearing loss

Result type: Positive, Possibly Positive, or Negative

Summary of causative variant(s)

Recommendations such as parental testing

RESULTS: PRIMARY VARIANTS

POSITIVE: A variant was identified in *GJB2*. These results are consistent with autosomal recessive non-syndromic hearing loss at the DFNB1 locus.

	Gene	Genomic Coordinates (hg38)	HGVS Nomenclature	Phenotype	Zygosity
Pathogenic	*GJB2*	chr13:g.20189473C>T	NM_004004.6: c.109G>A, p.(Val37Ile)	autosomal recessive non-syndromic hearing loss at the DFNB1 locus	Hom (100%)

RECOMMENDATIONS

- These results should be interpreted in the context of this individual's clinical findings and family history.
- Genetic counseling is recommended to help explain these results to this person and their family.

3 Interpretation of Primary Variants

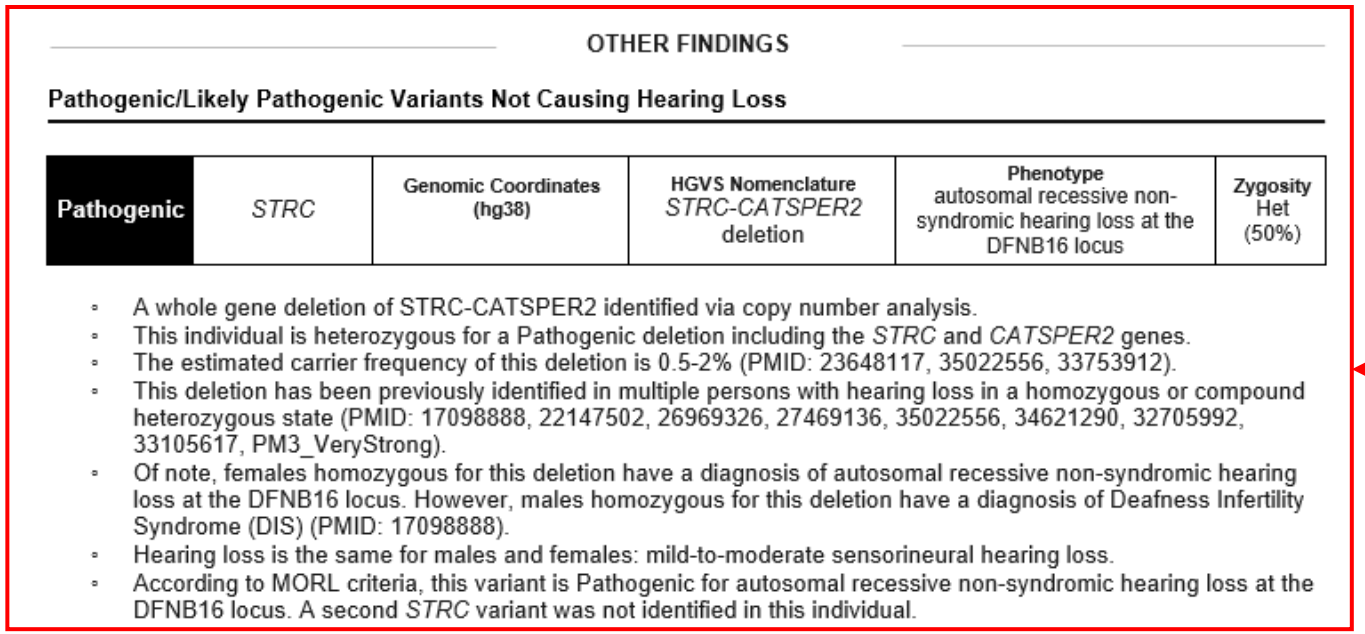
An in-depth analysis of the causative variant (if any) will be at the top of the Interpretation section

INTERPRETATION: PRIMARY VARIANTS

Pathogenic	*GJB2*	**Genomic Coordinates (hg38)** chr13:g.20189473C>T	**HGVS Nomenclature** NM_004004.6: c.109G>A, p.(Val37Ile)	**Phenotype** autosomal recessive non-syndromic hearing loss at the DFNB1 locus	**Zygosity** Hom (100%)

- Missense variant with a maximum minor allele frequency of 8.345% in the East Asian population as reported in the Genome Aggregation Database. There are 99 homozygotes reported.
- Genotype-phenotype correlation for DFNB1 hearing loss has been previously described (PMID: 16380907). These studies indicate persons homozygous for this variant in *GJB2* have mild-to-moderate autosomal recessive non-syndromic hearing loss (PMID: 16380907).
- The ClinGen Hearing Loss Expert Panel has published a consensus interpretation for the p.(Val37Ile) variant as Pathogenic for autosomal recessive nonsyndromic hearing loss with variable expressivity and incomplete penetrance (PMID: 31160754).
- Computational analysis predicts this missense variant is a substitute of a conserved variant (PhyloP, GERP++) and is predicted to be damaging (Polyphen-2, LRT, MutationTaster) with a CADD score of 22.5 (PP3).
- According to MORL criteria, this variant is **Pathogenic** for autosomal recessive non-syndromic hearing loss at the DFNB1 locus.

4 Pathogenic Variants Not Causing Hearing Loss



OTHER FINDINGS

Pathogenic/Likely Pathogenic Variants Not Causing Hearing Loss

Pathogenic	*STRC*	**Genomic Coordinates (hg38)**	**HGVS Nomenclature** *STRC-CATSPER2* deletion	**Phenotype** autosomal recessive non-syndromic hearing loss at the DFNB16 locus	**Zygosity** Het (50%)

- A whole gene deletion of STRC-CATSPER2 identified via copy number analysis.
- This individual is heterozygous for a Pathogenic deletion including the *STRC* and *CATSPER2* genes.
- The estimated carrier frequency of this deletion is 0.5-2% (PMID: 23648117, 35022556, 33753912).
- This deletion has been previously identified in multiple persons with hearing loss in a homozygous or compound heterozygous state (PMID: 17098888, 22147502, 26969326, 27469136, 35022556, 34621290, 32705992, 33105617, PM3_VeryStrong).
- Of note, females homozygous for this deletion have a diagnosis of autosomal recessive non-syndromic hearing loss at the DFNB16 locus. However, males homozygous for this deletion have a diagnosis of Deafness Infertility Syndrome (DIS) (PMID: 17098888).
- Hearing loss is the same for males and females: mild-to-moderate sensorineural hearing loss.
- According to MORL criteria, this variant is Pathogenic for autosomal recessive non-syndromic hearing loss at the DFNB16 locus. A second *STRC* variant was not identified in this individual.

If other Pathogenic/Likely Pathogenic variants that **do not** cause hearing loss are identified (i.e., carrier variants), an analysis of the variant will be at the top of the “Other Findings” section

Any variants that are not responsible for hearing loss but could be causative of another medical condition (secondary findings)

MORL
Molecular Otolaryngology & Renal Research Laboratories

5 Variants of Uncertain Significance

MORL | IOWA HEALTH CARE

OtoSCOPE® v9

Name, Patient

MORL ID: CDS-12345

Variants of Uncertain Significance (VUS)

VUS	*TNC*	Genomic Coordinates (hg38) chr9:g.115086109C>T	HGVS Nomenclature NM_002160.4: c.1622G>A, p.(Arg541His)	Phenotype Autosomal dominant nonsyndromic hearing loss 56	Zygosity Het (46%)

- A missense variant with a maximum minor allele frequency of 0.016% in the East Asian population as reported in the Genome Aggregation Database. There are 0 homozygotes reported.
- This variant has not been previously reported in persons with hearing loss.
- Computational analysis predicts this missense variant is a substitute of a conserved variant (GERP++) and is predicted to be damaging (SIFT, Polyphen-2, LRT, MutationTaster) with a CADD score of 23.6 (PP3).
- ***TNC*-related hearing loss is reported as postlingual, progressive, autosomal dominant hearing loss with an upsloping audiogram (PMID: 23936043). This person's audiogram does not fit *TNC*-related hearing loss and therefore is not thought to be causative of their hearing loss.**
- According to MORL criteria, this variant is of Uncertain Significance for autosomal dominant non-syndromic hearing loss at the DFNA56 locus. According to MORL criteria, this variant is of Uncertain Significance for autosomal dominant non-syndromic hearing loss at the DFNA56 locus.

Detailed interpretation of VUS

Bold text emphasizes key information, such as the rationale for excluding VUS as causative of hearing loss.

6 Variants of Uncertain Significance

Summary table of single VUS in in autosomal recessive phenotypes

This person also has single variants of uncertain significance (VUS) in the autosomal recessive genes below:

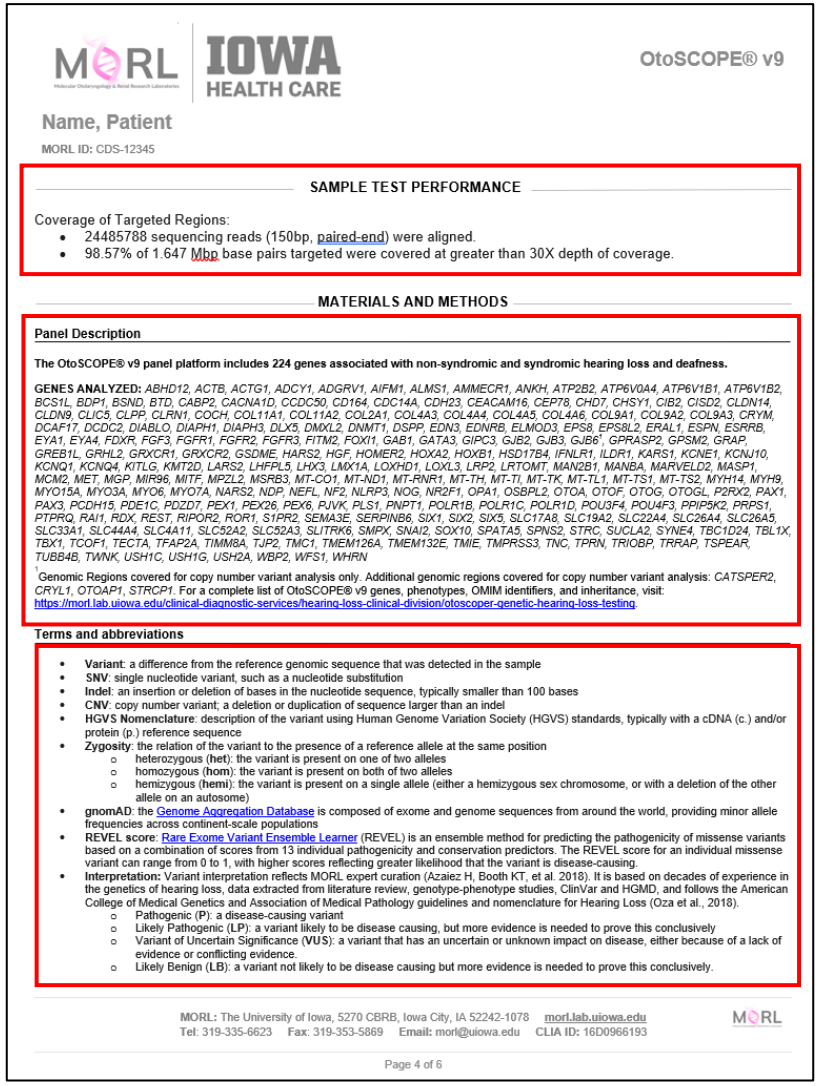
Interpretation	Gene	Genomic coordinates (GRCh387)	Transcript: Variant	Zygosity	gnomAD Frequency (%)	gnomAD Max (%)	REVEL
VUS	*SLC52A2*	chr8:g.144360652C>A	NM_001363118.2: c.1064C>A, p.(Ala355Glu)	Het (48%)	0.0009973	0.0004113	0.433
VUS	*USH2A*	chr1:g.215845838G>T	NM_206933.4: c.9041C>A, p.(Thr3014Asn)	Het (49%)	0.0028506	0.0010444	0.166

Should you have any questions regarding the interpretation of these data or should you wish to discuss these results in the context of this individual's phenotype, please contact our team.

7 Sample Performance and Materials and Methods

After the main report, there are several pages with detailed information on testing details and MORL policies.

List of all genes covered on the OtoSCOPE panel.
For more information on phenotypes, OMIM identifiers, and inheritance patterns can be found by clicking on the link



MORL | IOWA HEALTH CARE — OtoSCOPE® v9

Name, Patient

MORL ID: CDS-12345

SAMPLE TEST PERFORMANCE

Coverage of Targeted Regions:
- 24485788 sequencing reads (150bp, paired-end) were aligned.
- 98.57% of 1.647 Mbp base pairs targeted were covered at greater than 30X depth of coverage.

MATERIALS AND METHODS

Panel Description

The OtoSCOPE® v9 panel platform includes 224 genes associated with non-syndromic and syndromic hearing loss and deafness.

GENES ANALYZED: *ABHD12, ACTB, ACTG1, ADCY1, ADGRV1, AIFM1, ALMS1, AMMECR1, ANKH, ATP2B2, ATP6V0A4, ATP6V1B1, ATP6V1B2, BCS1L, BDP1, BSND, BTD, CABP2, CACNA1D, CCDC50, CD164, CDC14A, CDH23, CEACAM16, CEP78, CHD7, CHSY1, CIB2, CISD2, CLDN14, CLDN9, CLIC5, CLPP, CLRN1, COCH, COL11A1, COL11A2, COL2A1, COL4A3, COL4A4, COL4A5, COL4A6, COL9A1, COL9A2, COL9A3, CRYM, DCAF17, DCDC2, DIABLO, DIAPH1, DIAPH3, DLX5, DMXL2, DNMT1, DSPP, EDN3, EDNRB, ELMOD3, EPS8, EPS8L2, ERAL1, ESPN, ESRRB, EYA1, EYA4, FDXR, FGF3, FGFR1, FGFR2, FGFR3, FITM2, FOXI1, GAB1, GATA3, GIPC3, GJB2, GJB3, GJB6*[1], *GPRASP2, GPSM2, GRAP, GREB1L, GRHL2, GRXCR1, GRXCR2, GSDME, HARS2, HGF, HOMER2, HOXA2, HOXB1, HSD17B4, IFNLR1, ILDR1, KARS1, KCNE1, KCNJ10, KCNQ1, KCNQ4, KITLG, KMT2D, LARS2, LHFPL5, LHX3, LMX1A, LOXHD1, LOXL3, LRP2, LRTOMT, MAN2B1, MANBA, MARVELD2, MASP1, MCM2, MET, MGP, MIR96, MITF, MPZL2, MSRB3, MT-CO1, MT-ND1, MT-RNR1, MT-TH, MT-TI, MT-TK, MT-TL1, MT-TS1, MT-TS2, MYH14, MYH9, MYO15A, MYO3A, MYO6, MYO7A, NARS2, NDP, NEFL, NF2, NLRP3, NOG, NR2F1, OPA1, OSBPL2, OTOA, OTOF, OTOG, OTOGL, P2RX2, PAX1, PAX3, PCDH15, PDE1C, PDZD7, PEX1, PEX26, PEX6, PJVK, PLS1, PNPT1, POLR1B, POLR1C, POLR1D, POU3F4, POU4F3, PPIP5K2, PRPS1, PTPRQ, RAI1, RDX, REST, RIPOR2, ROR1, S1PR2, SEMA3E, SERPINB6, SIX1, SIX2, SIX5, SLC17A8, SLC19A2, SLC22A4, SLC26A4, SLC26A5, SLC33A1, SLC44A4, SLC4A11, SLC52A2, SLC52A3, SLITRK6, SMPX, SNAI2, SOX10, SPATA5, SPNS2, STRC, SUCLA2, SYNE4, TBC1D24, TBL1X, TBX1, TCOF1, TECTA, TFAP2A, TIMM8A, TJP2, TMC1, TMEM126A, TMEM132E, TMIE, TMPRSS3, TNC, TPRN, TRIOBP, TRRAP, TSPEAR, TUBB4B, TWNK, USH1C, USH1G, USH2A, WBP2, WFS1, WHRN*

[1]Genomic Regions covered for copy number variant analysis only. Additional genomic regions covered for copy number variant analysis: *CATSPER2, CRYL1, OTOAP1, STRCP1*. For a complete list of OtoSCOPE® v9 genes, phenotypes, OMIM identifiers, and inheritance, visit: https://morl.lab.uiowa.edu/clinical-diagnostic-services/hearing-loss-clinical-division/otoscoper-genetic-hearing-loss-testing.

Terms and abbreviations

- **Variant**: a difference from the reference genomic sequence that was detected in the sample
- **SNV**: single nucleotide variant, such as a nucleotide substitution
- **Indel**: an insertion or deletion of bases in the nucleotide sequence, typically smaller than 100 bases
- **CNV**: copy number variant; a deletion or duplication of sequence larger than an indel
- **HGVS Nomenclature**: description of the variant using Human Genome Variation Society (HGVS) standards, typically with a cDNA (c.) and/or protein (p.) reference sequence
- **Zygosity**: the relation of the variant to the presence of a reference allele at the same position
  - heterozygous (**het**): the variant is present on one of two alleles
  - homozygous (**hom**): the variant is present on both of two alleles
  - hemizygous (**hemi**): the variant is present on a single allele (either a hemizygous sex chromosome, or with a deletion of the other allele on an autosome)
- **gnomAD**: the Genome Aggregation Database is composed of exome and genome sequences from around the world, providing minor allele frequencies across continent-scale populations
- **REVEL score**: Rare Exome Variant Ensemble Learner (REVEL) is an ensemble method for predicting the pathogenicity of missense variants based on a combination of scores from 13 individual pathogenicity and conservation predictors. The REVEL score for an individual missense variant can range from 0 to 1, with higher scores reflecting greater likelihood that the variant is disease-causing.
- **Interpretation:** Variant interpretation reflects MORL expert curation (Azaiez H, Booth KT, et al. 2018). It is based on decades of experience in the genetics of hearing loss, data extracted from literature review, genotype-phenotype studies, ClinVar and HGMD, and follows the American College of Medical Genetics and Association of Medical Pathology guidelines and nomenclature for Hearing Loss (Oza et al., 2018).
  - Pathogenic (**P**): a disease-causing variant
  - Likely Pathogenic (**LP**): a variant likely to be disease causing, but more evidence is needed to prove this conclusively
  - Variant of Uncertain Significance (**VUS**): a variant that has an uncertain or unknown impact on disease, either because of a lack of evidence or conflicting evidence.
  - Likely Benign (**LB**): a variant not likely to be disease causing but more evidence is needed to prove this conclusively.

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Sample-specific test performance necessary for quality control

Definitions and abbreviations used in report

MORL
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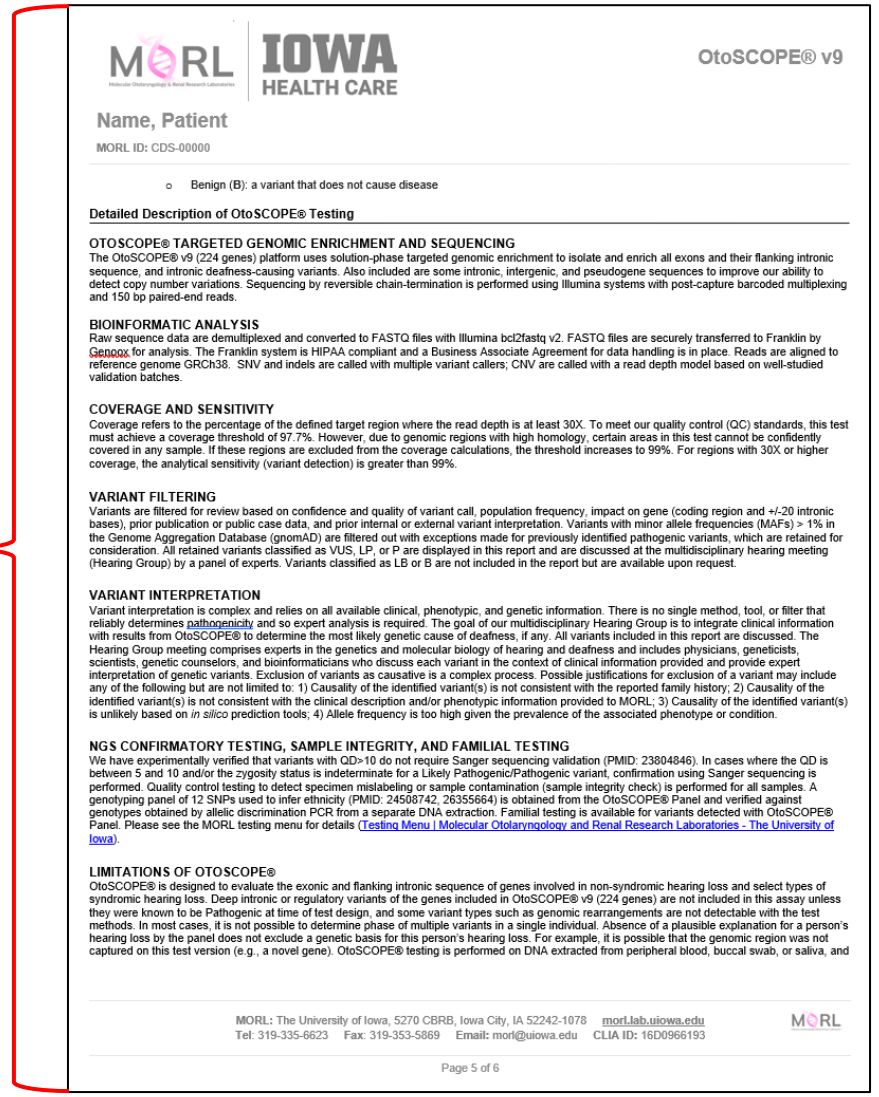
7 Sample Performance and Materials and Methods

Detailed materials and methods (genetic panel design, bioinformatic analysis, and variant filtering).



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OtoSCOPE® v9

Name, Patient

MORL ID: CDS-00000

- Benign (B): a variant that does not cause disease

Detailed Description of OtoSCOPE® Testing

OTOSCOPE® TARGETED GENOMIC ENRICHMENT AND SEQUENCING

The OtoSCOPE® v9 (224 genes) platform uses solution-phase targeted genomic enrichment to isolate and enrich all exons and their flanking intronic sequence, and intronic deafness-causing variants. Also included are some intronic, intergenic, and pseudogene sequences to improve our ability to detect copy number variations. Sequencing by reversible chain-termination is performed using Illumina systems with post-capture barcoded multiplexing and 150 bp paired-end reads.

BIOINFORMATIC ANALYSIS

Raw sequence data are demultiplexed and converted to FASTQ files with Illumina bcl2fastq v2. FASTQ files are securely transferred to Franklin by Genoox for analysis. The Franklin system is HIPAA compliant and a Business Associate Agreement for data handling is in place. Reads are aligned to reference genome GRCh38. SNV and indels are called with multiple variant callers; CNV are called with a read depth model based on well-studied validation batches.

COVERAGE AND SENSITIVITY

Coverage refers to the percentage of the defined target region where the read depth is at least 30X. To meet our quality control (QC) standards, this test must achieve a coverage threshold of 97.7%. However, due to genomic regions with high homology, certain areas in this test cannot be confidently covered in any sample. If these regions are excluded from the coverage calculations, the threshold increases to 99%. For regions with 30X or higher coverage, the analytical sensitivity (variant detection) is greater than 99%.

VARIANT FILTERING

Variants are filtered for review based on confidence and quality of variant call, population frequency, impact on gene (coding region and +/-20 intronic bases), prior publication or public case data, and prior internal or external variant interpretation. Variants with minor allele frequencies (MAFs) > 1% in the Genome Aggregation Database (gnomAD) are filtered out with exceptions made for previously identified pathogenic variants, which are retained for consideration. All retained variants classified as VUS, LP, or P are displayed in this report and are discussed at the multidisciplinary hearing meeting (Hearing Group) by a panel of experts. Variants classified as LB or B are not included in the report but are available upon request.

VARIANT INTERPRETATION

Variant interpretation is complex and relies on all available clinical, phenotypic, and genetic information. There is no single method, tool, or filter that reliably determines pathogenicity and so expert analysis is required. The goal of our multidisciplinary Hearing Group is to integrate clinical information with results from OtoSCOPE® to determine the most likely genetic cause of deafness, if any. All variants included in this report are discussed. The Hearing Group meeting comprises experts in the genetics and molecular biology of hearing and deafness and includes physicians, geneticists, scientists, genetic counselors, and bioinformaticians who discuss each variant in the context of clinical information provided and provide expert interpretation of genetic variants. Exclusion of variants as causative is a complex process. Possible justifications for exclusion of a variant may include any of the following but are not limited to: 1) Causality of the identified variant(s) is not consistent with the reported family history; 2) Causality of the identified variant(s) is not consistent with the clinical description and/or phenotypic information provided to MORL; 3) Causality of the identified variant(s) is unlikely based on *in silico* prediction tools; 4) Allele frequency is too high given the prevalence of the associated phenotype or condition.

NGS CONFIRMATORY TESTING, SAMPLE INTEGRITY, AND FAMILIAL TESTING

We have experimentally verified that variants with QD>10 do not require Sanger sequencing validation (PMID: 23804846). In cases where the QD is between 5 and 10 and/or the zygosity status is indeterminate for a Likely Pathogenic/Pathogenic variant, confirmation using Sanger sequencing is performed. Quality control testing to detect specimen mislabeling or sample contamination (sample integrity check) is performed for all samples. A genotyping panel of 12 SNPs used to infer ethnicity (PMID: 24508742, 26355664) is obtained from the OtoSCOPE® Panel and verified against genotypes obtained by allelic discrimination PCR from a separate DNA extraction. Familial testing is available for variants detected with OtoSCOPE® Panel. Please see the MORL testing menu for details (Testing Menu | Molecular Otolaryngology and Renal Research Laboratories - The University of Iowa).

LIMITATIONS OF OTOSCOPE®

OtoSCOPE® is designed to evaluate the exonic and flanking intronic sequence of genes involved in non-syndromic hearing loss and select types of syndromic hearing loss. Deep intronic or regulatory variants of the genes included in OtoSCOPE® v9 (224 genes) are not included in this assay unless they were known to be Pathogenic at time of test design, and some variant types such as genomic rearrangements are not detectable with the test methods. In most cases, it is not possible to determine phase of multiple variants in a single individual. Absence of a plausible explanation for a person's hearing loss by the panel does not exclude a genetic basis for this person's hearing loss. For example, it is possible that the genomic region was not captured on this test version (e.g., a novel gene). OtoSCOPE® testing is performed on DNA extracted from peripheral blood, buccal swab, or saliva, and

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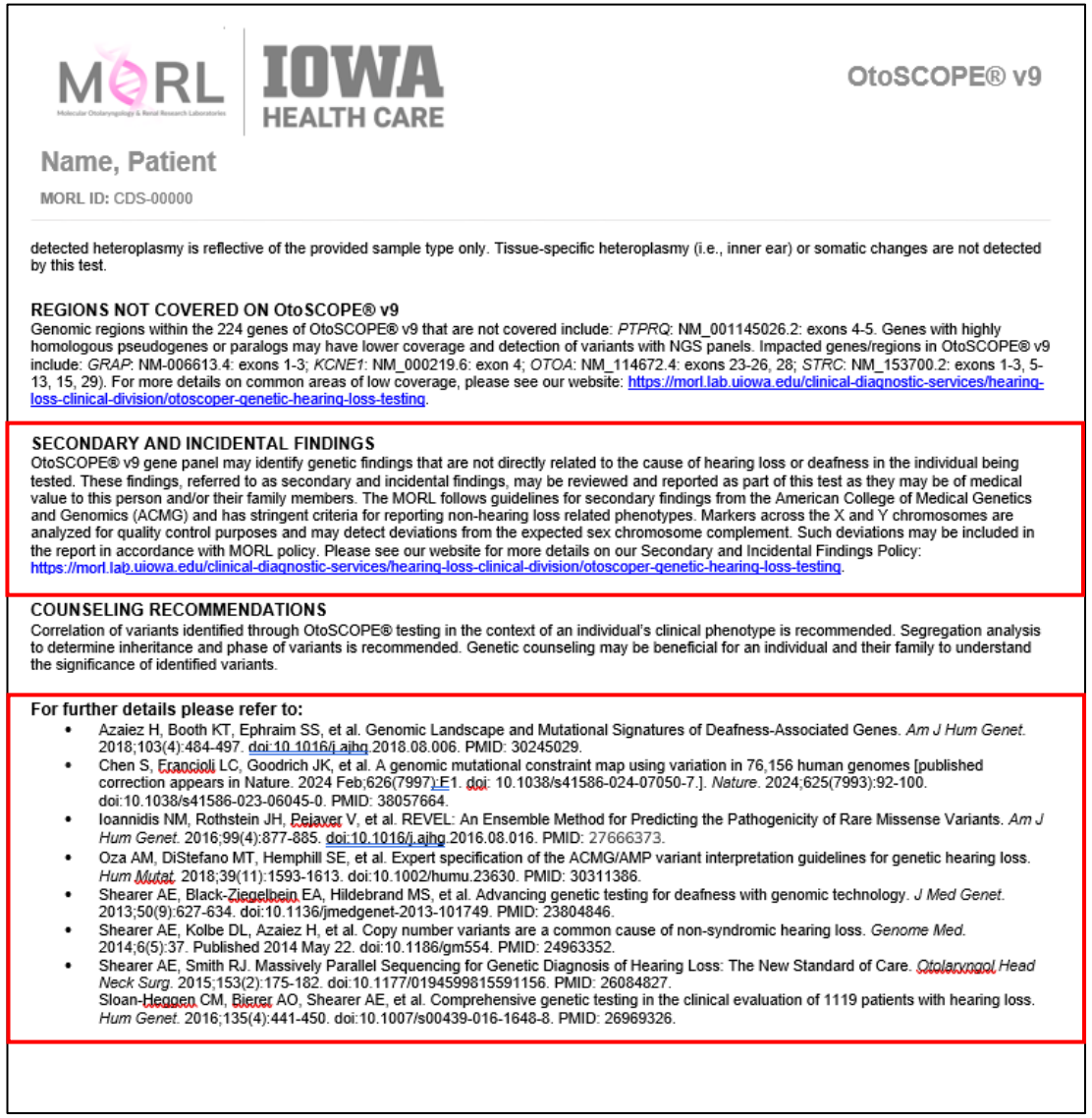
7 Sample Performance and Materials and Methods



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OtoSCOPE® v9

Name, Patient

MORL ID: CDS-00000

detected heteroplasmy is reflective of the provided sample type only. Tissue-specific heteroplasmy (i.e., inner ear) or somatic changes are not detected by this test.

REGIONS NOT COVERED ON OtoSCOPE® v9

Genomic regions within the 224 genes of OtoSCOPE® v9 that are not covered include: *PTPRQ*: NM_001145026.2: exons 4-5. Genes with highly homologous pseudogenes or paralogs may have lower coverage and detection of variants with NGS panels. Impacted genes/regions in OtoSCOPE® v9 include: *GRAP*: NM-006613.4: exons 1-3; *KCNE1*: NM_000219.6: exon 4; *OTOA*: NM_114672.4: exons 23-26, 28; *STRC*: NM_153700.2: exons 1-3, 5-13, 15, 29). For more details on common areas of low coverage, please see our website: https://morl.lab.uiowa.edu/clinical-diagnostic-services/hearing-loss-clinical-division/otoscoper-genetic-hearing-loss-testing.

SECONDARY AND INCIDENTAL FINDINGS

OtoSCOPE® v9 gene panel may identify genetic findings that are not directly related to the cause of hearing loss or deafness in the individual being tested. These findings, referred to as secondary and incidental findings, may be reviewed and reported as part of this test as they may be of medical value to this person and/or their family members. The MORL follows guidelines for secondary findings from the American College of Medical Genetics and Genomics (ACMG) and has stringent criteria for reporting non-hearing loss related phenotypes. Markers across the X and Y chromosomes are analyzed for quality control purposes and may detect deviations from the expected sex chromosome complement. Such deviations may be included in the report in accordance with MORL policy. Please see our website for more details on our Secondary and Incidental Findings Policy: https://morl.lab.uiowa.edu/clinical-diagnostic-services/hearing-loss-clinical-division/otoscoper-genetic-hearing-loss-testing.

COUNSELING RECOMMENDATIONS

Correlation of variants identified through OtoSCOPE® testing in the context of an individual's clinical phenotype is recommended. Segregation analysis to determine inheritance and phase of variants is recommended. Genetic counseling may be beneficial for an individual and their family to understand the significance of identified variants.

For further details please refer to:

- Azaiez H, Booth KT, Ephraim SS, et al. Genomic Landscape and Mutational Signatures of Deafness-Associated Genes. *Am J Hum Genet*. 2018;103(4):484-497. doi:10.1016/j.ajhg.2018.08.006. PMID: 30245029.
- Chen S, Francioli LC, Goodrich JK, et al. A genomic mutational constraint map using variation in 76,156 human genomes [published correction appears in Nature. 2024 Feb;626(7997):E1. doi: 10.1038/s41586-024-07050-7.]. *Nature*. 2024;625(7993):92-100. doi:10.1038/s41586-023-06045-0. PMID: 38057664.
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- Oza AM, DiStefano MT, Hemphill SE, et al. Expert specification of the ACMG/AMP variant interpretation guidelines for genetic hearing loss. *Hum Mutat*. 2018;39(11):1593-1613. doi:10.1002/humu.23630. PMID: 30311386.
- Shearer AE, Black-Ziegelbein EA, Hildebrand MS, et al. Advancing genetic testing for deafness with genomic technology. *J Med Genet*. 2013;50(9):627-634. doi:10.1136/jmedgenet-2013-101749. PMID: 23804846.
- Shearer AE, Kolbe DL, Azaiez H, et al. Copy number variants are a common cause of non-syndromic hearing loss. *Genome Med*. 2014;6(5):37. Published 2014 May 22. doi:10.1186/gm554. PMID: 24963352.
- Shearer AE, Smith RJ. Massively Parallel Sequencing for Genetic Diagnosis of Hearing Loss: The New Standard of Care. *Otolaryngol Head Neck Surg*. 2015;153(2):175-182. doi:10.1177/0194599815591156. PMID: 26084827.
  Sloan-Heggen CM, Bierer AO, Shearer AE, et al. Comprehensive genetic testing in the clinical evaluation of 1119 patients with hearing loss. *Hum Genet*. 2016;135(4):441-450. doi:10.1007/s00439-016-1648-8. PMID: 26969326.

A description of the MORL's policy on secondary and incidental findings. More information can be found on the MORL website.

References

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