Supp. Figure S1. Example report. This report was generated using the GeneInsight system.

HARVARD MEDICAL SCHOOL PARTNERS HEALTHCARE

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Lab Accession: [redacted]
Patient Name: [redacted]
Birth Date: [redacted]
Age Sex: [redacted]

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MOLECULAR DIAGNOSTICS REPORT

Specimen Type:	Blood, Peripheral	**Report Date:**	[redacted]
Related Accession(s):	[redacted]	**Received Date:**	[redacted]
Referring Physician:	[redacted]	**Referring Facility:**	[redacted]
Copies To:	[redacted]	**Referring Fac. MRN:**	[redacted]
		Lab Control Number:	[redacted]

TEST PERFORMED - Cxn-pnlA; Oto-pnlA

TEST DESCRIPTION - Connexin Test (GJB2 Sequencing and GJB6-D13S1830 Deletion)
OtoChip for Hearing Loss and Usher Syndrome (19 Genes Sequenced)

INDICATION FOR TEST - Congenital profound sensorineural hearing loss with hypothyroidism and a family history of hearing loss

RESULTS

DNA VARIANTS:
Heterozygous c.1001+1G>A, Intron 8, SLC26A4, Pathogenic
Heterozygous c.2188C>T (p.Gln730X), Exon 19, SLC26A4, Pathogenic

INTERPRETATION:
Positive.

OtoChip Test: Positive. DNA sequencing of 19 genes associated with hearing loss and related syndromes identified the variants listed above. No clinically significant variants were detected in the other 17 genes. See Methodology below for information on the specific genes and regions sequenced.

The **1001+1G>A** variant has been previously described in the literature with well established pathogenicity (Coyle 1998, Campbell 2001, Bogazzi 2000, Lopez-Bigas 2001, Fugazzola 2002, Bogazzi 2004, Pryor 2005). This variant is predicted to cause abnormal splicing because the nucleotide substitution occurs in the invariant region of the splice consensus sequence.

The **Gln730X** variant in SLC26A4 has not been reported in the literature nor previously identified by our laboratory. However, this variant leads to a premature stop codon at position 730, which is predicted to lead to a truncated or absent protein. Therefore, this variant meets our criteria to be classified as pathogenic.

In summary, it is highly likely that this individual's hearing loss and hypothyroidism are due to the 1001+1G>A and Gln730X SLC26A4 variants. Although there are no reports of these variants occurring in cis (on the same copy of the gene), testing of parental DNA would be needed to confirm that both copies of the gene are affected and no normal copy of the gene exists.

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Pendred syndrome and DFNB4 hearing loss are inherited in an autosomal recessive manner. Two carriers have a 25% (or 1 in 4) risk for having an affected child.

Connexin Test (GJB2 sequencing and GJB6-D13S1830 deletion): Negative. DNA sequencing of exon 1, the coding region of exon 2 and all splice junctions did not identify any clinically significant variants in GJB2 (Connexin 26). A PCR-based analysis of the GJB6-D13S1830 region of chromosome 13 was performed and did not detect the deletion.

RECOMMENDATION:
Testing of parents is recommended to assess for cis/trans configuration of the SLC26A4 variants in this individual.

Genetic counseling is recommended for this individual and their family members. Familial mutation testing is available for other family members if desired. For assistance in locating genetic counseling services or disease specialists please call the laboratory at 617-768-8500 or email at LMM@partners.org

COMMENTS:
Common sequence variants of unlikely clinical significance are not included in this report but are available upon request.

In addition, the following less common sequence variants have been identified. Although they are likely benign we cannot rule out that they may be pathogenic.

Val1734Met in exon 41 of CDH23: This variant has not been reported in the literature nor previously identified by our laboratory. Computational analyses (biochemical amino acid properties, homology, PolyPhen, SIFT, AlignGVGD) do not provide strong support for pathogenicity. In addition, this patient already has two pathogenic variants which sufficiently explain their hearing loss. In summary, this data suggests this variant is more likely benign.

Asp2936Asp in exon 61 of CDH23: This variant is not expected to have clinical significance because it does not alter an amino acid residue and is not located near a splice junction.

-6T>A in exon 2 of GJB2: This variant has been reported in the literature and is likely benign due to equal occurrence in cases and controls. It was found in 3 of 144 Black control chromosomes (Tang 2006) and 3 of 418 proband chromosomes from multiple or unspecified races (Tang 2006, Al-Qahtani, 2010, Shan 2010).

Thr2523Thr in exon 33 of GPR98: This variant is not expected to have clinical significance because it does not alter an amino acid residue and is not located near a splice junction.

Glu994Glu in exon 28 of MYO6: This variant is not expected to have clinical significance because it does not alter an amino acid residue and is not located near a splice junction. In addition, this variant has been reported as benign and is seen in an equal frequency in probands (1/50) and controls (3/120) (Ahituv 2000, rs55905349).

Ala918Ala in exon 23 of MYO7A: This variant is not expected to have clinical significance because it does not alter an amino acid residue and is not located near a splice junction.

Pro1341Pro in exon 31 of MYO7A: This variant is not expected to have clinical significance because it does not alter an amino acid residue, is not located near a

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splice junction and is listed in dbSNP in 2/50 chromosomes (rs73495790). In addition, this variant has previously been reported as benign because it was identified at a similar frequency in affected individuals (1/40) and in control subjects (1/28) (Jaijo 2007).

Ala357Thr in exon 9 of SLC26A4: This variant has not been reported in the literature nor previously identified by our laboratory. This residue is not highly conserved in mammals and computational analyses (PolyPhen, SIFT, AlignGVGD) do not suggest a high likelihood of impact to the protein. In addition, this patient already has two pathogenic variants which sufficiently explain their hearing loss. In summary, this data suggests this variant is more likely benign.

Val217Val in exon 8 of USH1C: This variant is not expected to have clinical significance because it does not alter an amino acid residue and is not located near a splice junction.

Lys130Glu in exon 2 of USH1G: This variant is not expected to have clinical significance due to an equal occurrence in probands (1/109) and controls (1/100) (Aller 2007).

Glu142Lys in exon 2 of USH1G: This variant is not expected to have clinical significance due to an equal occurrence in probands (1/109) and controls (1/100) (Aller 2007).

Cys2123Cys in exon 33 of USH2A: This variant is not expected to have clinical significance because it does not alter an amino acid residue and is not located near a splice junction.

INCIDENTAL VARIANTS:
Heterozygous c.5200G>A (p.Val1734Met), Exon 41, CDH23, Likely Benign
Heterozygous c.8808C>T (p.Asp2936Asp), Exon 61, CDH23, Likely Benign
Heterozygous c.-6T>A (5'UTR), Intron 1, GJB2, Likely Benign
Heterozygous c.7569A>G (p.Thr2523Thr), Exon 33, GPR98, Likely Benign
Heterozygous c.2982G>A (p.Glu994Glu), Exon 28, MYO6, Likely Benign
Heterozygous c.2754C>T (p.Ala918Ala), Exon 23, MYO7A, Likely Benign
Heterozygous c.4023C>T (p.Pro1341Pro), Exon 31, MYO7A, Likely Benign
Heterozygous c.1069G>A (p.Ala357Thr), Exon 9, SLC26A4, Likely Benign
Heterozygous c.651A>G (p.Val217Val), Exon 8, USH1C, Likely Benign
Heterozygous c.388A>G (p.Lys130Glu), Exon 2, USH1G, Likely Benign
Heterozygous c.424G>A (p.Glu142Lys), Exon 2, USH1G, Likely Benign
Heterozygous c.6369C>T (p.Cys2123Cys), Exon 33, USH2A, Likely Benign

TEST INFORMATION

BACKGROUND:
Over 50% of isolated childhood hearing loss has a genetic etiology, with over 50 genes involved. Hereditary hearing loss is most commonly inherited in an autosomal recessive pattern, however, some genes are inherited in autosomal dominant, X-linked, or mitochondrial pattern. For specific information the genetics of nonsyndromic hearing loss please see our website (http://www.hpcgg.org/LMM) or Smith and Camp (2007). Usher syndrome is a condition characterized by sensorineural hearing loss, retinitis pigmentosa and variable vestibular dysfunction. At least 9 identified genes are responsible for the three clinical subtypes. Some of these genes can also cause isolated hearing loss. Usher syndrome is inherited in an autosomal recessive pattern.

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For additional information on Usher syndrome please see our website (http://www.hpcgg.org/LMM) or Keats and Lentz (2008) for Type 1, Keats and Lentz (2007) for Type 2, or Cohen (2007) for Type 3.

METHODOLOGY:
For the Connexin Test exon 1 and the coding region of exon 2 of the connexin 26 (GJB2) gene (NM_004004) are amplified using flanking primer sets. PCR products are sequenced using an ABI fluorescence automatic DNA sequencer. This test does not detect large deletions or mutations in non-coding regions that could affect gene expression. This assay is greater than 99.9% accurate in detecting mutations in the sequences analyzed. Polymerase chain reaction (PCR) analysis is performed to detect the presence or absence of a deletion spanning the GJB6-D13S1830 region of chromosome 13.

The OtoChip test is performed by a combination of oligonucleotide hybridization-based DNA sequencing and dideoxy-based DNA sequencing of the coding regions and splice sites of CDH23 (NM_022124.2), CLRN1 (NM_174878.2, NM_174880.1, NM_052995.2), DFNB31 (NM_015404.1), exon 2 (excludes 35delG) of GJB2 (NM_004004) plus the -3202+1G>A exon 1 splice site variant, exon 3 of GJB6 (Connexin 30) (NM_006783.4), exons 8, 20, 31-41 and 89 of GPR98 (NM_032119.3), exons 2-35 of MYO6 (NM_004999.3), exons 2-49 of MYO7A (NM_000260), OTOF (NM_194248.1) with 2 exons from alternative transcripts (NM_194323.1 and AF183183), exons 2-33 of PCDH15 (NM_033056.3), SLC26A4 (NM_000441.1) plus the -103T>C exon 1 variant, exons 5-24 of TMC1 (NM_138691.2), TMIE (NM_147196.1), exons 2-13 of TMPRSS3 (NM_024022.1), USH1C (NM_153676.2) with 1 exon from an alternative transcript (NM_005709.2), USH1G (NM_173477.2), USH2A (NM_206933.1 and NM_007123.4), and MTTS1 as well as genotyping of mutations 961delT, 961T>C, 961T>G, 1095T>C, 1494T>C, 1555A>G in MTRNR1.

The OtoChip is at least 97% sensitive for detecting substitution variants in the sequence analyzed. This test does not examine most non-coding regions that could affect gene expression. Compared to dideoxy sequencing this test has significantly reduced sensitivity (37%) for detecting small insertions and deletions (indels), except for 281 previously identified indels for which genotyping probes have been included. These indels can be detected at 95% sensitivity. Like traditional sequencing tests, the OtoChip has very little sensitivity for detecting large deletions, insertions and other copy number changes.

These tests were developed and the performance characteristics determined by the Laboratory for Molecular Medicine at the Partners Healthcare Center for Personalized Genetic Medicine (LMM, 65 Landsdowne St, Cambridge, MA 02139; 617-768-8500; CLIA#22D1005307). It has not been cleared or approved by the U.S Food and Drug Administration (FDA). The FDA has determined that such clearance or approval is not necessary.

Variants are reported according to HGVS nomenclature (www.hgvs.og/mutnomen).

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