athena diagnostics

Hearing Loss Advanced Sequencing and CNV Evaluation

Test Code: 3029

Specimen Requirements: 8 mL room temperature or refrigerated whole blood collected in 2 EDTA (lavender-top) tubes; 6 mL minimum

CPT Codes*: 81430; 81431

CLINICAL USE

Identify the cause of genetic hearing loss

CLINICAL BACKGROUND

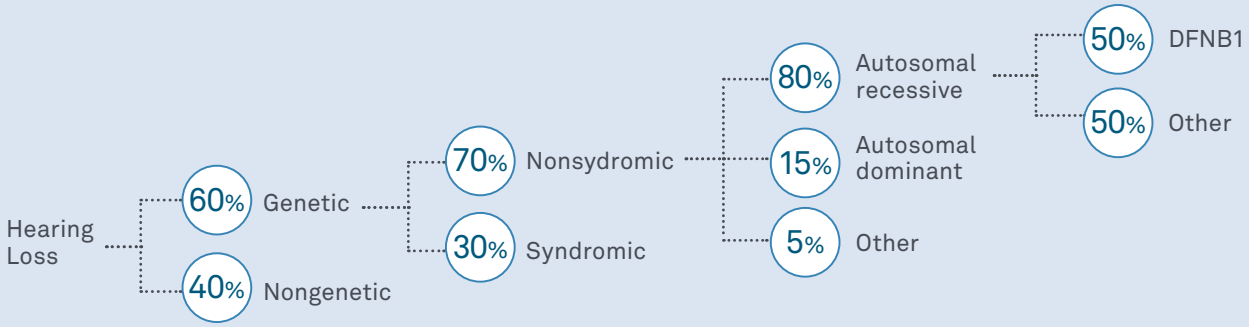
Identifying the cause of congenital deafness and hearing loss has important implications for prognosis and treatment; however, identification can be challenging because of the many potential etiologies.[1] An initial assessment of hearing loss should involve audiometric tests, physical and neurological examination, and gathering patient, birth, and family histories.[1] Based on the assessment, a clinician can decide if the cause is more likely to be nongenetic (eg, infectious diseases such as meningitis or rubella) or genetic. Acquired nongenetic causes account for 40% of all hearing loss (Figure 1). If a nongenetic cause is suspected, appropriate tests, such as testing for infectious disease (eg, cytomegalovirus) or imaging, should be conducted. Genetic causes account for 60% of all hearing loss (Figure 1).

Genetic hearing loss can be generally categorized as syndromic (associated with defects in other organs) or nonsyndromic, but differentiating the categories can be difficult.[1] Over 400 syndromes are associated with hearing loss, and those syndromes account for 30% of genetic hearing loss. Some of the more common syndromes, depending on the population, include Pendred, Usher, Waardenburg, and branchio-oto-renal syndromes. Over 100 genes are associated with nonsyndromic hearing loss, which accounts for 70% of genetic hearing loss. Genetic hearing loss can also be categorized by inheritance pattern (eg, autosomal recessive, autosomal dominant, sex-linked, mitochondrial) (Figure 1).[1]

Genetic testing for hearing loss

The benefits of genetic testing include shortening the diagnostic course and guiding therapy, counseling, and education. Genetic testing can also help avoid hearing loss in family members who are susceptible to certain drugs; for example, aminoglycoside treatment can cause hearing loss in individuals with mutations in the MT-RNR1 gene. Furthermore, testing can confirm syndromic conditions, which may indicate a need for surveillance for defects in other organs. Though genetic testing is an important diagnostic tool, it does not render nongenetic tests unnecessary, as they can guide genetic testing. One example is the use of temporal bone imaging; if an enlarged vestibular aqueduct is identified, genetic testing for Pendred syndrome may be warranted.[1]

Figure 1: hearing loss categories and proportions



This figure was developed by Quest Diagnostics based in part on reference 1. It is provided for informational purposes only and is not intended as medical advice. A physician's test selection and interpretation, diagnosis, and patient management decisions should be based on his/her education, clinical expertise, and assessment of the patient.

A key differentiator of the many available tests for genetic hearing loss is the scope of genetic targets. Targets range from specific variants or genes to the whole exome or genome. Each approach has advantages and disadvantages that should be considered.

- A narrow approach, such as single-gene testing, may be cost-effective, but it could extend the time to diagnosis. Such an approach may be appropriate if clinical evidence indicates a specific gene or syndrome.[1]
- The broadest approaches are whole-exome sequencing (WES) or whole-genome sequencing (WGS), which are unbiased approaches to identify potential causative variants. However, these approaches can lead to incidental findings and difficulties in interpreting uncharacterized variants. These methods may also be limited by technical challenges and fail to efficiently identify hearing loss-related deletions, duplications, or copy number variants.[2]
- A middle ground is a multigene panel that targets genes or variants associated with hearing loss. This approach avoids the limitations of WES and WGS. The disadvantages of this approach are that it may cost more than single-gene testing and it is limited to what is currently known about genetic hearing loss.[1]

Guidelines from the American College of Medical Genetics and Genomics

The American College of Medical Genetics and Genomics (ACMG) provides general guidance on genetic testing related to hearing loss.[1] The guidelines emphasize that pretest genetic counseling should be provided before testing. They also emphasize that clinical evidence should guide which testing method is used.

If syndromic hearing loss is suspected, genetic testing should be ordered for confirmation. Targeted gene testing should be considered, but confirmation testing may include a narrow (ie, single-gene) or broader approach (ie, multigene panel, WES, WGS), depending on clinical evidence.[1] If nonsyndromic hearing loss is suspected, ACMG recommends a tiered genetic testing approach. However, the guidelines note that next-generation sequencing is quickly driving down the cost of testing; thus, using a hearing loss-targeted, multigene panel as the initial test may be more cost-effective than a tiered approach.[1]

For the tiered approach, single-gene testing may be appropriate as a first step if a specific etiology is suspected.[1] As an example, DFNB1 testing is recommended if 1) specific clinical indications are absent, 2) autosomal recessive inheritance is observed, or 3) the case is a single occurrence in a family. Variants in the DFNB1 locus, which contains 2 genes associated with hearing loss (GJB2 and GJB6) that encode connexins, represent the most common cause of genetic hearing loss. DFNB1-related hearing loss is inherited in an autosomal recessive pattern and accounts for half of autosomal recessive nonsyndromic hearing loss (15%-40% of all hearing loss, depending on the population).[1] If mutations in DFNB1 are not identified, ACMG recommends considering WES, WGS, or a panel that targets hearing loss-specific genes.

Genetic tests for hearing loss etiologies

Athena Diagnostics offers the Hearing Loss Advanced Sequencing and CNV Evaluation, a next-generation sequencing test that detects genetic defects associated with hearing loss. These include sequence variants and copy number variations in 183 genes, the DFNB1 locus, and the overlapping MT-TI/MT-TQ (tRNA) sequence (Table 1). Genes are included in the panel only if hearing loss has been confirmed in at least 2 patients or a well-pedigreed family. Athena Diagnostics also offers separate tests for the connexin genes located in the DFNB1 locus: test code 321 for connexin 26 (GJB2), test code 319 for connexin 30 (GJB6), and test code 329 for both genes.

INDIVIDUALS SUITABLE FOR TESTING

Individuals with suspected genetic hearing loss

METHOD

The Hearing Loss Advanced Sequencing and CNV Evaluation has a sensitivity of ~99% and specificity is ~99% for identifying DNA sequencing variants and copy number variants (CNVs).

The assay uses next-generation sequencing (NGS) to identify DNA variants by sequencing and CNVs. The assay has a mean coverage depth of 30 sequencing reads; regions with <20 sequencing reads may not be reported. Sanger sequencing may be used to identify sequencing variants in regions that are difficult to sequence by NGS. Targeted microarray or multiplex ligation-dependent probe amplification (MLPA) using genomic DNA may be performed to confirm CNV results.

The Hearing Loss Advanced Sequencing and CNV Evaluation includes analysis for 183 genes, the DFNB1 locus, and the overlapping MT-TI/MT-TQ (tRNA) sequence (Table 1):

- CNV analysis for the DFNB1 locus and GJB2

- Sequencing analysis for 11 mitochondrial genes, the overlapping MT-TI/MT-TQ (tRNA) sequence, and SALL1
- Both CNV analysis and sequencing analysis for the exomes of 170 other hearing loss-related genes

REFERENCE RANGE

Not detected

INTERPRETIVE INFORMATION

The presence of a pathogenic variant associated with hearing loss indicates hearing loss may be caused by the variant.

A negative result does not rule out a genetic etiology; variants can occur in genes or gene regions that are not included in this test.[1] Benign and likely benign variants, if identified, are considered normal and are not reported but are available upon request.

Additional assistance in interpretation of results is available from our genetic counselors and scientific and medical staff by calling 1.866.GENE.INFO (1.866.436.3463).

References

1. Alford RL, Arnos KS, Fox M, et al. American College of Medical Genetics and Genomics guideline for the clinical evaluation and etiologic diagnosis of hearing loss. *Genet Med*. 2014;16:347-355.
2. Rehm HL, Bale SJ, Bayrak-Toydemir P, et al. ACMG clinical laboratory standards for next-generation sequencing. *Genet Med*. 2013;15:733-747.

Table 1: Genes Analyzed in the Hearing Loss Advanced Sequencing and CNV Evaluation[a]

ABHD12	*CDH23*	*COL9A3*	*ESRRB*	*HSD17B4*	*MT-RNR1 (rRNA)*[c]	*NR2F1*	*RDX*	*SYNE4*
ACTB	*CEACAM16*	*CRYM*	*EYA1*	*ILDR1*	*MT-TH (tRNA)*[c]	*OPA1*	*RIPOR2 (FAM65B)*	*TBC1D24*
ACTG1	*CEMIP*	*DCDC2*	*EYA4*	*KARS*	*MT-TI (tRNA)*[c]	*OSBPL2*	*ROR1*	*TBX1*
ADCY1	*CHD7*	*DFNA5*	*FGF3*	*KCNE1*	*MT-TI / MT-TQ (tRNA)*[c]	*OTOA*	*S1PR2*	*TCOF1*
ADGRV1 (GPR98)	*CHSY1*	*DFNB1 locus*[b]	*FGFR1*	*KCNJ10*	*MT-TK (tRNA)*[c]	*OTOF*	*SALL1*[c]	*TECTA*
AIFM1	*CIB2*	*DFNB59 (PJVK)*	*FGFR2*	*KCNQ1*	*MT-TL1 (tRNA)*[c]	*OTOG*	*SEMA3E*	*TFAP2A*
ALMS1	*CISD2*	*DIABLO*	*FGFR3*	*KCNQ4*	*MT-TQ (tRNA)*[c]	*OTOGL*	*SERPINB6*	*TIMM8A*
ANKH	*CLDN14*	*DIAPH1*	*FOXI1*	*LARS2*	*MT-TS1 (tRNA)*[c]	*P2RX2*	*SIX1*	*TJP2*
ATP2B2 (PMCA2)	*CLIC5*	*DIAPH3*	*GATA3*	*LHFPL5*	*MT-TS2 (tRNA)*[c]	*PAX3*	*SIX5*	*TMC1*
ATP6V1B1	*CLPP*	*DLX5*	*GIPC3*	*LOXHD1*	*MYH14*	*PCDH15*	*SLC12A1*	*TMEM132E*
ATP6V1B2	*CLRN1*	*DNMT1*	*GJB2*	*LRTOMT*	*MYH9*	*PDZD7*	*SLC17A8*	*TMIE*
BCS1L	*COCH*	*DSPP*	*GJB3*	*MANBA*	*MYO15A*	*PEX1*	*SLC19A2*	*TMPRSS3*
BDP1	*COL11A1*	*EDN3*	*GJB6*[b]	*MARVELD2*	*MYO3A*	*PEX6*	*SLC22A4*	*TNC*
BSND	*COL11A2*	*EDNRA*	*GPSM2*	*MCM2*	*MYO6*	*PMP22*	*SLC26A4*	*TPRN*
CABP2	*COL2A1*	*EDNRB*	*GRHL2*	*MET*	*MYO7A*	*PNPT1*	*SLC26A5*	*TRIOBP*
CACNA1D	*COL4A3*	*ELMOD3*	*GRXCR1*	*MIR96 (miRNA)*	*NARS2*	*POLR1C*	*SLC4A11*	*TSPEAR*
CCDC50	*COL4A4*	*EPS8*	*GRXCR2*	*MITF*	*NDP*	*POLR1D*	*SLITRK6*	*USH1C*
CD151	*COL4A5*	*EPS8L2*	*HARS2*	*MSRB3*	*NLRP3*	*POU3F4*	*SMPX*	*USH1G*
CD164	*COL4A6*	*ERCC2*	*HGF*	*MT-CO1*[c]	*PTPRQ*	*POU4F3*	*SNAI2*	*USH2A*
CDC14A	*COL9A1*	*ERCC3*	*HOMER2*	*MT-CO2*[c]		*PRPS1*	*SOX10*	*WFS1*
	COL9A2	*ESPN*	*HOXB1*	*MT-ND1*[c]			*STRC*	*WHRN*

[a] Sequencing is not available for all individual genes

[b] CNV analysis only, ie, no sequencing analysis

[c] Sequencing analysis only, ie, no CNV analysis

* The CPT codes provided are based on AMA guidelines and are for informational purposes only. CPT coding is the sole responsibility of the billing party. Please direct any questions regarding coding to the payer being billed.

This test was developed and its performance characteristics determined by Athena Diagnostics. It has not been cleared or approved by the FDA. The laboratory is regulated under CLIA as qualified to perform high-complexity testing. This test is used for clinical purposes. It should not be regarded as investigational or for research.