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ACMG PRACTICE RESOURCE

Clinical evaluation and etiologic diagnosis of hearing loss: A clinical practice resource of the American College of Medical Genetics and Genomics (ACMG)

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ABSTRACT

Hearing loss is a common and complex condition that can occur at any age, can be inherited or acquired, and is associated with a remarkably wide array of etiologies. The diverse causes of hearing loss, combined with the highly variable and often overlapping presentations of different forms of hearing loss, challenge the ability of traditional clinical evaluations to arrive at an etiologic diagnosis for many deaf and hard-of-hearing individuals. However, identifying the etiology of hearing loss may affect clinical management, improve prognostic accuracy, and refine genetic counseling and assessment of the likelihood of recurrence for relatives of deaf and hard-of-hearing individuals. Linguistic and cultural identities associated with being deaf or hard-of-hearing can complicate access to and the effectiveness of clinical care. These concerns can be minimized when genetic and other health care services are provided in a linguistically and culturally sensitive manner. This clinical practice resource offers information about the frequency, causes, and presentations of hearing loss and suggests approaches to the clinical and genetic evaluation of deaf and hard-of-hearing individuals aimed at identifying an etiologic diagnosis and providing informative and effective patient education and genetic counseling.

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Definitions

Deaf: a community with a distinct culture and visual language shaped by the experience of being deaf or hard of hearing, which may include deaf, hard-of-hearing, and hearing individuals

deaf: an auditory phenotype characterized by a total or near-total loss of the ability to hear

hard-of-hearing: an auditory phenotype characterized by a partial loss of the ability to hear

hearing loss: an auditory phenotype characterized by any degree of loss of the ability to hear; depending on the cause, hearing loss can be temporary or permanent—this practice resource focuses on permanent hearing loss

Introduction

Two to 3 of every 1000 children born in the United States are deaf or have hearing loss (HL) significant enough to affect speech and language development.[1] Early intervention has been shown to be effective in facilitating speech and language development in deaf and hard-of-hearing children.[2] As a result, newborn hearing screening (NBHS), which began in 2001, is now mandated at the state level throughout the United States. However, not all childhood HL is present at birth, and hearing screening is recommended throughout childhood and adolescence to identify children with later-onset HL and to permit early intervention.[3,4]

Ninety-five percent of newborns with HL identified by NBHS programs are born to hearing parents, obscuring the fact that most newborns have a hereditary cause for their HL.[5,6] Analysis of family history data from school-aged children in the United States estimated that up to 60% of educationally significant congenital and early-onset HL is caused by genetic factors.[5,6] Most genetic HL is inherited in an autosomal recessive pattern and often presents in the absence of a positive family history for HL. One gene, *GJB2*, which encodes the gap junction protein connexin 26, accounts for the largest proportion of autosomal recessive early childhood HL in many populations.[7]

The prevalence of HL increases with age, with 40% to 50% of the population experiencing HL by age 75.[8] The contribution of genetic causes to cases of adult-onset HL is less clear. However, it is evident that a significant proportion of adult-onset HL is likely to be caused, or strongly influenced, by genetic factors.[9-14]

The goal of a genetics evaluation for deaf and hard-of-hearing individuals of any age is to identify an etiologic diagnosis and, in doing so, enable implementation of an individualized health maintenance strategy.[15-17] Identification of a previously unrecognized syndromic form of HL can be particularly important because it may allow early management of associated medical concerns. Obtaining an etiologic diagnosis also provides the basis for precise genetic counseling that includes an accurate estimation of the chances for recurrence of HL within families. There is increasing awareness of the importance of an early etiologic diagnosis as outlined in a recent proposal for a comprehensive tiered testing approach in newborns.[18] Because the cost of next-generation sequencing (NGS) is rapidly decreasing, NGS-based large gene panels have become the choice of testing for HL.[19] At least 18 HL-focused NGS panels are registered in the Genetic Testing Registry (GTR) (https://www.ncbi.nlm.nih.gov/gtr/tests/), with an average of 121 genes per panel (range = 23-308).[20] The diagnostic yield of comprehensive HL NGS panels that cover all genes known to be associated with nonsyndromic HL and more common syndromic HL is approximately 40%.[21] Genome-wide sequencing such as exome sequencing (ES) and genome sequencing (GS) have reported diagnostic yields between 30% and 35% for ES.[22,23]

In 2014, the American College of Medical Genetics and Genomics (ACMG) published a practice guideline for the clinical evaluation and etiologic diagnosis of HL.[24] The document was intended to offer information about the frequency, causes, and clinical presentations of HL; suggest approaches to clinical evaluation; provide methods of etiologic diagnosis; and emphasize the significance of informative and effective patient education and genetic counseling. The ACMG Professional Practice and Guidelines Committee reviewed the documents and solicited input from the original authors. The committee considers that the original guideline still represents the current clinical practice in general; however, the emergence of NGS technologies and associated bioinformatics tools has led to a rapid expansion of our knowledge regarding the genetic etiology of HL. Therefore, instead of adding an addendum to the original document, a workgroup was formed to update the guideline to a clinical practice resource that reflects the current knowledge, especially in the areas of genetic testing, gene–disease relationship, and variant curation specific to HL. An updated algorithm for the clinical and diagnostic evaluation of HL is also proposed.

Methods

In 2013, a working group of the ACMG developed an initial practice guideline for the clinical evaluation and etiologic diagnosis of HL. This was based on expert opinions of professionals working in the fields of medical genetics, otolaryngology, audiology, genetic counseling, genetic testing, and HL research. Recommendations were supported by the biomedical literature where available. As part of the regular review program of ACMG documents, a new workgroup was established to review and update the existing document. An initial addendum was drafted; however, the working group decided to integrate the updated elements into the original document to create a single up-to-date resource. This updated document was finalized through

iterative review and editing between the workgroup members. A draft document was posted on the ACMG website, and an email link was sent inviting ACMG members to provide comments. All comments were assessed by the authors, and when appropriate, changes were made to address member comments. Both member comments and workgroup responses were reviewed by the ACMG Board of Directors, and the final document was approved by the ACMG Board of Directors.

Audiometric and Clinical Aspects of HL

HL is typically described in terms related to its clinical presentation. In general, HL is categorized as either syndromic or nonsyndromic, depending on the presence or absence of involvement in other organ systems. HL is also typically described by the following:

- Age of onset: congenital, prelingual (before the acquisition of speech), postlingual (after the acquisition of speech), adult onset, or presbycusis (age-related late-onset HL)
- Type of HL: sensorineural, conductive, mixed, or auditory neuropathy
- Laterality and symmetry of HL: unilateral or bilateral, symmetric or asymmetric
- Stability of HL: progressive, nonprogressive, or fluctuating
- Degree of HL: slight (16-25 decibels [dB]), mild (26-40 dB), moderate (41-55 dB), moderately severe (56-70 dB), severe (71-90 dB), or profound (91 dB or greater)[25]
- Configuration of HL as seen on audiometric analysis: sloping, flat, rising (low frequency), or midfrequency (cookie-bite) loss

HL may also be described according to an apparent pattern of inheritance—autosomal recessive, autosomal dominant, X-linked, or matrilineal (mitochondrial). If a specific etiology is known, descriptions of HL may also include the etiologic diagnosis, such as Usher syndrome type 1B–related HL or *GJB2*-related HL.[15,16,26,27]

Genetic and Nongenetic Etiologies of HL

HL is among the most etiologically heterogeneous disorders, with more than 400 genetic syndromes that include HL as a feature, more than 100 genes associated with nonsyndromic genetic HL, and a number of nongenetic causes.[27,28] Genes associated with syndromic and nonsyndromic genetic HL encode a variety of proteins involved in the development and function of the auditory system, including transcription factors, structural proteins, gap junction proteins, and ion channels, to name just a few.[29]

An estimated 30% of genetic HL is syndromic. A few syndromes, such as Pendred (enlarged vestibular aqueduct, thyroid problems), Usher (retinitis pigmentosa), Waardenburg (pigmentary anomalies), and branchiootorenal (branchial arch and renal anomalies) syndromes, account for substantial percentages of HL in some populations.[27,30-33] Syndromic HL may be transmitted as an autosomal recessive, autosomal dominant, X-linked, or matrilineal trait. A review of individual conditions can be found in *Hereditary Hearing Loss and Its Syndromes* by Toriello and Smith[27] and the online database GeneReviews.[26]

For some syndromic forms of HL, such as Usher syndrome, Jervell and Lange-Nielsen syndrome, or Pendred syndrome, the nonauditory features can be subtle or even absent in early childhood. These conditions can present challenging clinical and counseling issues.[34-36] For others, HL is not the presenting finding or the most pressing concern. For many syndromic forms of HL, there is marked variability in the phenotypic presentation and age of onset of syndromic features. This variability can exist both between and within families. For example, HL is observed in only about 50% of individuals with Waardenburg syndrome, the specific likelihood being dependent on the molecular/clinical subtype.[37] As a result, this diagnosis can be easily missed if specific information about pigmentary changes or gastrointestinal disturbances is not elicited.[38] Branchiootorenal syndrome is another example with marked variability, where the condition is often diagnosed when a family member presents with HL and/or ear anomalies. Furthermore, some hereditary forms of HL, such as neurofibromatosis type 2, enlarged vestibular aqueduct syndrome, and Pendred syndrome, may present initially as unilateral HL.[26,27,39-41] Given the challenges that can exist in distinguishing between syndromic and nonsyndromic forms of HL, all children and adolescents showing HL without a known etiology, eg, confirmed pathogenic *GJB2* variants, should be evaluated for syndromic conditions by a clinical geneticist.[15,16]

An estimated 70% of genetic HL is nonsyndromic. Nonsyndromic HL may be transmitted as an autosomal recessive (~80%), autosomal dominant (~15%), or X-linked trait (~1%).[27] In addition, matrilineal (mitochondrial) transmission of nonsyndromic HL occurs with a frequency of approximately 1% in Western nations but has a slightly higher incidence in Spain and East Asian countries, including China, Mongolia, Korea, and Japan.[42,43]

Although nonsyndromic HL demonstrates high genetic heterogeneity, the DFNB1 locus, which includes the *GJB2* gene encoding the gap junction protein connexin 26 and the *GJB6* gene encoding the gap junction protein connexin 30, accounts for an estimated 50% of all autosomal recessive nonsyndromic HL and 15% to 40% of all deaf individuals in a variety of populations.[7,44-50] More than 150 deafness-causing variants have been identified in *GJB2*, but a few common variants account for a large percentage of alleles in several populations.[7,46-48] *GJB2*-related HL is sensorineural, usually present at birth, and typically bilateral and

nonprogressive and can range from mild to profound in severity. However, progressive or later-onset HL—with infants passing their newborn hearing screen—have also been described, particularly in association with non-truncating variants.[51-54] A combination of (1) a pathogenic *GJB2* variant on 1 allele and a deletion involving *GJB6* on the other allele or (2) biallelic deletions involving *GJB6* have been associated with nonsyndromic HL.[55-57] These *GJB6* deletions are suspected to result in HL owing to a regulatory effect on the expression of *GJB2*.[55,58] *GJB6* deletions have been observed in multiple populations, although they appear to be a relatively uncommon explanation for HL in the United States.[59-62] Notably, HL caused by certain dominant variants in *GJB2*, although uncommon, may present as syndromic HL, with associated skin findings.[63-65] Recent data also suggest that alterations in the stereocilin (*STRC*) gene represent the next most common genetic etiology, accounting for roughly 30% of patients with mild to moderate HL, making it the most common etiology for this group. It accounted for 16% among all groups. Interestingly, most variants were large copy number variants (CNVs), emphasizing the importance of CNV detection in any HL gene panel.[35,66,67] It is worth noting that *STRC* sequence variants may be underdetected because of the presence of the pseudogene.

Nonsyndromic mitochondrial HL is characterized by audiograms that fall into the moderate-to-profound range and is associated with variants in either the *MT-RNR1* gene, encoding mitochondrial 12S ribosomal RNA, or the *MT-TS1* gene, encoding the mitochondrial transfer RNA Ser(UCN).[42,43,68] Of particular note, pathogenic variants in *MT-RNR1* are associated with predisposition to aminoglycoside ototoxicity.[46] HL in individuals exposed to aminoglycoside antibiotics who carry pathogenic variants in *MT-RNR1* is bilateral, severe to profound, and typically develops within a few days to weeks after administration of any amount, including just a single dose, of an aminoglycoside antibiotic.[69] The Clinical Pharmacogenetics Implementation Consortium recently recommended that use of aminoglycosides should be avoided in individuals with an *MT-RNR1* variant associated with an increased risk of aminoglycoside-induced HL, unless the high risk of permanent HL is outweighed by the severity of infection and safe or effective alternative therapies are not available.[70] Studies offer conflicting findings with regard to the likelihood of HL in individuals carrying a pathogenic variant in *MT-RNR1* who are not exposed to aminoglycosides.[46,69,71] HL can also be part of the clinical presentation for some syndromic mitochondrial disorders, such as maternally inherited diabetes and deafness, which often has high-frequency HL. Testing mitochondrial genome plus deletion analysis for these disorders is indicated in the appropriate clinical setting, eg, diabetes with high-frequency HL and/or a maternal family history of HL and/or diabetes.[72]

Age-related HL, or presbycusis, is a common neurosensory deficit. In the United States, presbycusis is present in 40% to 50% of individuals aged 75 and older. Presbycusis generally affects higher frequencies of sound disproportionately, making it difficult for those with presbycusis to understand speech.[8] Men have presbycusis more frequently than women.[73] Presbycusis is a complex condition influenced by genetic and environmental factors.[14] Much of the literature about age-related HL has focused on environmental factors, such as noise exposure.[9,74,75] More recently, however, several susceptibility loci for age-related HL have been identified. Genes implicated in this process using linkage and genome-wide association studies include genes previously implicated in other forms of HL (such as *KCNQ4* and *ACTG1*) and genes involved in oxidative stress (such as *GRM7*, *GRHL2*, mitochondrial oxidative genes, and *N*-acetyltransferase).[9,10,12-14,27,76] A recent study also showed that ultrarare heterozygous pathogenic variants of genes causing dominant forms of early-onset deafness may underlie severe presbycusis.[77]

It is often difficult to discern between autosomal dominant genetic HL and presbycusis in the absence of a family history of HL. It is also important to consider that the age of onset of HL may be difficult to distinguish from the age at which HL loss is recognized and diagnosed, particularly for individuals born before widespread newborn and childhood hearing screening. Comparison of audiometric data of adults with HL to the presbycusis-hearing threshold standard, ISO 7029, can assist clinicians in differentiating possible adult-onset Mendelian HL from presbycusis.[76] Studies estimating genetic testing diagnostic yield for adult-onset HL is between 18% and 35%.[35,78,79] Therefore, although the yield for late-onset HL is lower compared with congenital or childhood onset, offering genetic testing for later-onset HL is reasonable to identify an etiology.[76]

Similarly, unilateral HL can present a diagnostic challenge because it may progress to bilateral HL, represent a nongenetic condition such as congenital cytomegalovirus (cCMV), or be caused by a condition that involves an inner ear malformation or cochlear nerve anomaly. The diagnostic yield of NGS HL gene panel testing for unilateral HL is around 1% to 5%, with most diagnoses caused by syndromic etiologies.[35,80] Genetic testing for unilateral HL should be considered to investigate possible genetic etiologies, particularly because syndromes can present with subtle findings. If nongenetic causes are ruled out, or if a genetic cause cannot be ruled out, NGS gene panel testing for HL is warranted.[81]

Certain environmental (nongenetic) factors play a major etiologic role in HL.[82] In the United States, cCMV infection is the most common nongenetic cause of HL among children. Of the 20,000 to 40,000 infants born with cCMV infection each year, 90% have no detectable clinical abnormalities at birth, yet 10% to 15% of these asymptomatic infants will develop sensorineural HL, which can present in early childhood, can be unilateral or bilateral, and is often progressive.[83-85] As a result, cCMV infection may go undetected even in children who undergo NBHS and receive a thorough physical examination in the neonatal period.[16,27,85] This association has led to the implementation of

hearing-targeted cCMV screening in a few states. Currently, Utah, Connecticut, Iowa, and Virginia have legislation to mandate cCMV screening after failed NBHS to identify HL associated with asymptomatic cCMV (Congenital CMV Legislation in the United States. National CMV Foundation. https://www.nationalcmv.org/about-us/advocacy). At present, the efficacy of antiviral treatment with ganciclovir/valganciclovir in mitigating HL from asymptomatic cCMV is still unclear, although there are a few ongoing clinical trials to help understand this better (https://clinicaltrials.gov/ct2/show/NCT03107871).[86]

Congenital rubella, which was a common cause of HL in the mid-1960s, occurs less frequently in Western populations today as a result of successful immunization programs.[87,88] According to the World Health Organization, no cases of endemic rubella infection are known to have occurred in the Americas between 2009 and 2012.[89] Similarly, the occurrence of postmeningitic HL in children has been substantially reduced in developed countries as a result of vaccination against *Haemophilus influenzae*, *Neisseria meningitidis*, and *Streptococcus pneumoniae*.[90] However, other environmental causes for HL, including prematurity and exposure to noise or ototoxic drugs such as aminoglycosides and cyclophosphamides (which may have a genetically determined predisposition in some cases), persist in the United States even today.[27,91-93]

Genetic Evaluation and Genetic Counseling for Deaf and Hard-of-Hearing Individuals

Because genetic etiology is likely in most infants and children with HL, clinical genetics evaluation, including genetic counseling, should be offered to every child with confirmed HL. Genetic counseling and genetic testing for HL offer a number of potential benefits for children and adults and their families. Benefits can include providing etiologic information, identifying (or allaying concerns about) comorbidities that may need referral for specialty care, planning for future medical and educational needs, facilitating estimations of the likelihood of recurrence, allowing families to better plan for the birth of a deaf or hard-of-hearing child, relieving the guilt that some parents may feel about having a child with HL, enhancing psychological well-being, dispelling misinformation, and facilitating identification and referral for unrelated hereditary conditions such as familial cancer.[17,61,94-101] Furthermore, if mitochondrial DNA variants associated with genetic susceptibility to aminoglycoside ototoxicity are discovered, it may be possible for relatives to avoid precipitating medications as a primary preventative measure.[46,69,71]

As with any genetics evaluation, clear communication between the genetics professionals and their patients is important for the provision of effective genetics services. Deaf and hard-of-hearing individuals use a variety of communication methods, including spoken and signed language, lip reading, and written notes. Special training may be needed to optimize communication between individuals with HL and genetics professionals. Such training may include (1) training sign language interpreters in medical and genetics terminology and (2) training genetics professionals to work effectively with sign language interpreters and use a variety of communication aids, including videophones, video relay services, instant messaging, and visual aids.[102]

In addition, deafness is considered by some to be a nonmedical trait. Many deaf individuals consider themselves to be part of a linguistic and cultural minority group, viewing their deafness as a neutral or positive trait.[103,104] By contrast, the medical perspective, which views deafness as a pathology, is pervasive among most hearing individuals and some deaf individuals. This difference in perspective may affect the willingness of some individuals to obtain genetic services and genetic counseling.[105,106] However, when given accurate information about the nature of genetic counseling and how to obtain a referral, deaf adults are often interested in receiving genetic services to learn more about themselves and why they are deaf or hard of hearing. In addition, many deaf and hard-of-hearing individuals report an enhanced sense of self-understanding and self-identity, as well as an enhanced cultural and group identity, as a result of genetic testing.[97,107] Providing genetic services in a linguistically and culturally sensitive manner has been shown to improve outcomes such as genetics knowledge and understanding.[108,109] Furthermore, using neutral or balanced terminology, such as chance instead of risk and deaf or hearing instead of affected or unaffected, and exercising caution in the use of terms such as handicapped, pathology, and impairment can enhance the provision of genetic services to deaf and hard-of-hearing individuals and their families.[108,110,111]

Pretest and posttest genetic counseling should be provided to facilitate informed decision making driven by patient and family goals and values, including the decision to not pursue genetic testing. Pretest counseling includes review of the potential benefits, risks, and limitations of genetic testing and possible test results, including potential diagnosis of a nonsyndromic mimic in an infant or child with HL. If ES or GS are discussed, pretest counseling must include review of potential incidental or secondary findings in accordance with ACMG practice recommendations.[112-114] Posttest genetic counseling is recommended regardless of genetic test outcome. For patients in whom genetic testing identifies an etiology for HL, posttest counseling should include prognostic information on HL stability vs progression, referrals to other medical specialties as indicated, discussion of support organizations and review of inheritance, and chance for future children with HL. For inconclusive results, variants of uncertain significance (VUS) should be reviewed to determine if genetic testing of additional relatives may resolve the significance of the VUS. For negative genetic test results, posttest counseling can include discussion of additional genetic or nongenetic

testing and evaluations and the importance of a return visit with genetics to assess for new genetic testing options.

Genetic Testing for the Etiologic Diagnosis of Hereditary HL

Historically, molecular diagnostic tests for HL have used genotyping or DNA sequencing to identify specific HL variants or to screen individual genes, or small collections of genes, for changes associated with HL. This approach has proven to be effective in cases in which there is a single gene, or limited number of genes, responsible for a subtype of HL. Examples include *SLC26A4* gene sequencing in individuals with cochlear malformations or otherwise suspected of having Pendred syndrome, *PAX3* gene sequencing in individuals with features of Waardenburg syndrome type I, *MITF* and *SOX10* gene sequencing in individuals with features of Waardenburg syndrome type II, or sequencing of *MYO7A* or *USH2A*, the most common genes involved in Usher syndrome types I and II, respectively.[115,116] Such screening can also be cost-effective in individuals with genetically heterogeneous HL phenotypes when a single gene is responsible for a significant percentage of cases. For example, *GJB2* gene sequencing can identify the underlying etiology for many individuals whose clinical presentation is consistent with autosomal recessive nonsyndromic HL.

Today, tests based on NGS technologies have replaced most single gene–sequencing tests for HL (Figure 1). These tests use disease-targeted exon-capture (targeted gene panel testing), ES, or GS strategies. The main advantage of these tests is their ability to address the problem of genetic heterogeneity, wherein variants in many different genes result in phenotypes that cannot be easily distinguished clinically.[26,117-120] The diagnostic yield of NGS-based testing for unilateral HL is low because of nongenetic causes; however, it is still the preferred method because of genetic heterogeneity.[81] Numerous NGS tests are now clinically available and can be found by querying the GeneTests and GTR websites.[20,26]

NGS tests that use disease-targeted exon-capture approaches restrict sequencing to specific genes, such as genes known to be associated with HL. Such tests can provide excellent coverage of the genes selected for study but are limited by our present knowledge of which genes are involved in HL. Furthermore, some tests may sequence only a subset of the genes known to be associated with HL. ES is also based on exon capture but does not rely on a list of genes involved in a particular disease process. Instead, ES seeks to evaluate all exons in the genome for variation. This approach can identify variants in known HL-related genes and genes that have yet to be associated with HL or genes associated with syndromes not suspected by the ordering health care provider. GS is not limited to screening exons and therefore has the potential to identify changes outside of exons that may be related to HL. ES/GS can detect variants in more genes but may have reduced analytical sensitivity in some genes as compared with a panel, where higher read depth and Sanger fill-in may be part of standard workflows to ensure adequate coverage and CNV analysis may be more reliably available.

The ability of ES and GS approaches to detect causative changes in all possible HL-associated genes needs to be balanced with the difficulties in interpretation that come from identifying tens of thousands to millions of variants per exome or genome, the challenge of causally linking variants in new genes to HL, and the likelihood of identifying genetic susceptibilities unrelated to HL (ie, incidental or secondary findings).[121] Beginning in 2013, ACMG has published several versions of recommendations for reporting secondary findings from genomic sequencing.[113,122,123]

Furthermore, not all regions of the genome are efficiently captured and analyzed by current exon-capture or GS approaches, and large deletions and duplications, in addition to copy number and structural variations, may not be efficiently detected.[121] These limitations of NGS technologies may necessitate use of alternative or complementary genetic testing strategies in some cases. For example, variants in the *STRC* gene, including single or multigene deletions, are a common cause of HL but have been less well studied owing to the technical challenges of the genomic locus, which includes a segmental duplication with a nearly identical pseudogene.[66]

NGS technologies and analytical methods have improved significantly over time, but it will always be important to pay close attention to the performance characteristics of tests, including test design, genomic regions covered (also known as regions of interest [ROIs]), technologies used, analytic sensitivity, and limitations of the test. Some panel tests are designed to cover every single nucleotide in the ROI, whereas others analyze a subset of ES data, where certain regions within the ROI may have low or no coverage unless these regions are specifically filled in with additional probes or by Sanger sequencing. Additionally, some panels include copy number analysis for detecting deletions/duplications in the ROI, whereas others may only interrogate sequence variants (single-nucleotide variants and insertion-deletions); therefore, additional deletion/duplication studies may need to be considered. Patients with syndromic HL with negative comprehensive gene panel results may be further evaluated with ES or GS; the latter has the benefit of examining large structural variations.[124] In some cases, it may be helpful to have tests performed in laboratories that focus on genetic causes of HL because these laboratories may be more likely to report test performance with respect to hearing-related genes and to have developed approaches to specifically analyze relevant regions of the genome that may be refractory to more general NGS approaches.[117-121,125]

More than 100 genes have been proposed to be associated with nonsyndromic HL, and more than 400 have been proposed to be associated with syndromic HL.[126] Establishing that a gene is associated with a disease is critical because ACMG/Association for Molecular Pathology

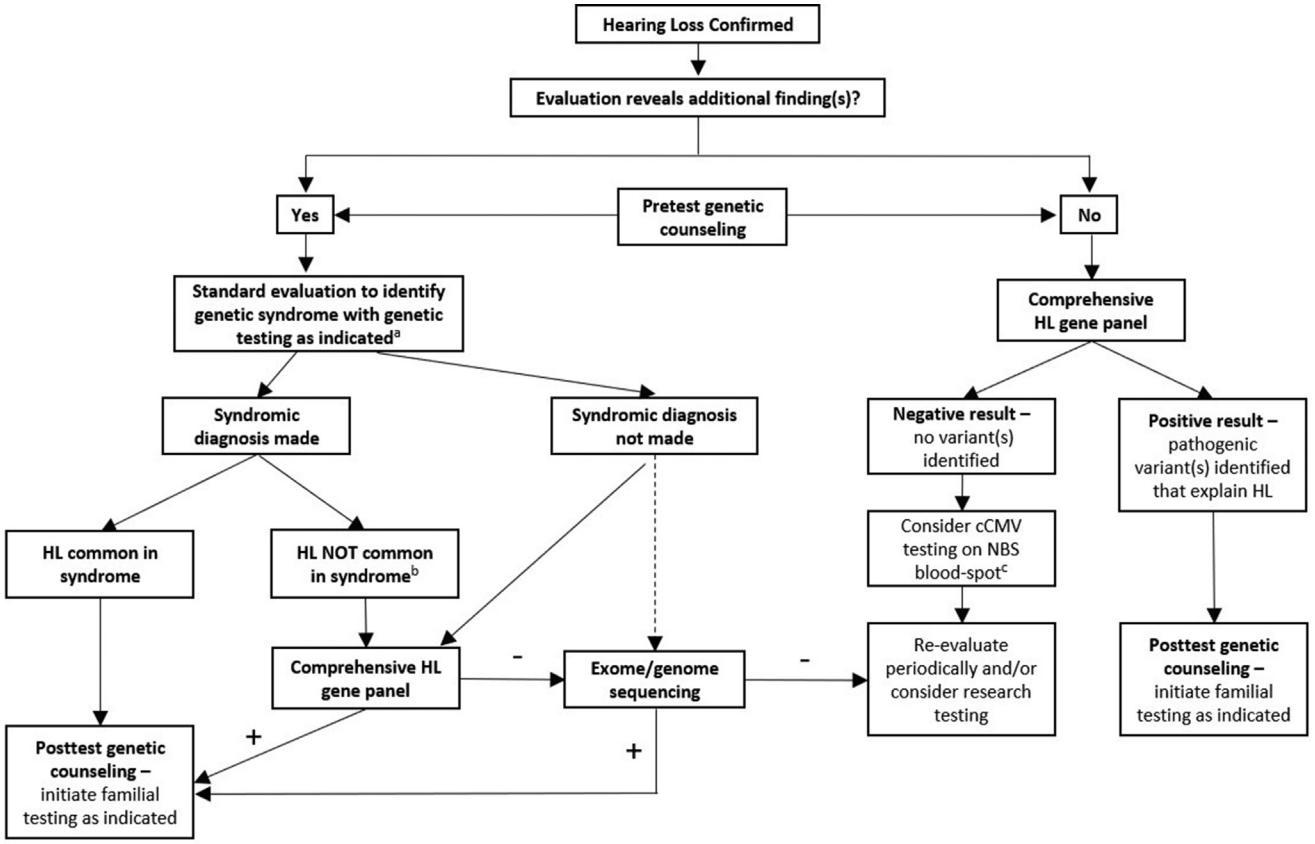

Figure 1 Approach to clinical and diagnostic evaluation for hearing loss. [a]Genetic testing could include single-gene tests, multigene panels, chromosome analysis, or microarray depending on clinical findings. [b]If genetic syndrome identified is not typically associated with HL, proceed to evaluate for secondary cause of HL. [c]Birth state may screen newborns for cCMV. The symbol + indicates positive. The symbol – indicates negative. cCMV, congenital cytomegalovirus; HL, hearing loss; NBS, newborn screening.

sequence variant and ACMG/Clinical Genome Resource (ClinGen) CNV interpretation guidelines suggest that genes should have a well-documented association with the disease before variant interpretation can be performed.[127,128] The National Institutes of Health–funded ClinGen has developed a semiquantitative framework to evaluate gene–disease relationships.[129,130] The HL Gene Curation Expert Panel (https://clinicalgenome.org/affiliation/40007/) was formed in 2016 and includes clinicians, laboratory geneticists, genetic counselors, and researchers. To date, the group has curated 164 gene–disease relationships from 142 unique genes that were retrieved from GTR with a primary focus on those conditions that could present as nonsyndromic HL. Of the 164 gene–disease pairs evaluated for an association, 82 were definitive, 12 were strong, 25 were moderate, 32 were limited, 10 were disputed, and 3 were refuted.[131] The group continues to meet on a quarterly basis to evaluate new gene–disease relationships or to reevaluate those that meet the time limit for recuration per ClinGen guidelines (https://clinicalgenome.org/curation-activities/gene-disease-validity/training-materials/#Documentation).[132] All gene curations, a summary paragraph, and the evidence used to score them are marked with the date of approval and are available on the ClinGen website (https://search.clinicalgenome.org/kb/gene-validity).

In addition to gene curation, the ClinGen HL Variant Curation Expert Panel (HL-VCEP) adapted the ACMG/Association for Molecular Pathology variant interpretation guideline[127] and specified its 28 benign and pathogenic rules, which are applied at different levels of strength (supporting, moderate, strong, very strong), to be used in the context of genetic HL and specifically in 9 representative genes: *USH2A*, *SLC26A4*, *GJB2*, *MYO7A*, *CDH23*, *TECTA*, *COCH*, *KCNQ4*, and *MYO6*. Overall, HL-VCEP specified 21 rules and removed 4 rules, whereas 3 rules remained unchanged.[133,134] The panel leveraged the expertise of its members to quantify specific genetic attributes of HL, such as prevalence, penetrance, inheritance patterns, and genetic and allelic heterogeneity, to specify the allele frequency rules (BA1, BS1, PM2), and to characterize frequency thresholds above which variants are considered benign or likely benign while taking into consideration well-known common founder variants in *GJB2* and *SLC26A4*.[133,135] In addition, knowledge about functional assays in HL genes was used to define the functional study codes (PS3, BS3), whereas gene-specific clinical presentations were used to modify the phenotype code (PP4). Furthermore, HL-VCEP worked closely with the ClinGen Sequence Variant Interpretation workgroup to refine several general rules, such as the loss-of-function (PVS1),[134] allelic (PM3), segregation

(PP1), and de novo (PM6, PS2) rules. In aggregate, the specified rules were tested and refined using 51 variants selected by the expert panel and were shown to change classification in 19 of the 51 variants (37%).[133] More recently, HL-VCEP showed that HL-specified rules led to unambiguous classifications (benign, likely benign, likely pathogenic, pathogenic) in 70% (109/157) of variants that previously were mostly VUS or had conflicting interpretations in ClinVar.[136] Given the complexity of genetic architecture underlying HL, the collective gene and variant curation efforts described above have helped standardize clinical interpretation of HL-associated sequence variants, which will ultimately lead to better management of individuals with HL.

Other Testing Important to the Etiologic Diagnosis of HL

Because CMV remains a common cause of pediatric HL, testing for cCMV infection by rapid culture or polymerase chain reaction (PCR) of saliva or urine samples from newborns is recommended as an initial test once newborn HL is confirmed (Figure 1).[137-141] The consensus recommendations from the International Congenital Cytomegalovirus Recommendations Group are that the diagnosis of cCMV infection in neonates should include real-time PCR of saliva, urine, or both, as soon as possible after birth but within the first 3 weeks of life, with saliva as the preferred sample.[142] For children in whom cCMV infection was not tested within the first 3 weeks of life, retrospective diagnosis using PCR analysis of newborn screening blood-spot cards may be available with relatively high sensitivity and specificity.[143] A negative result most likely excludes CMV as the cause of HL, but a positive result may not necessarily indicate that HL is caused by CMV infection, especially if obtained in older children who may have been exposed to CMV after birth.

Recent algorithms for the evaluation of HL suggest that other nongenetic tests, such as computed tomography and/or magnetic resonance imaging of the temporal bone, renal ultrasonography, electrocardiography, and ophthalmologic consultation, have an important role because their results can guide genetic testing or interpretation of DNA sequence variants.[144] For example, temporal bone imaging is commonly recommended to look for an enlarged vestibular aqueduct or other temporal bone anomalies, which would prompt genetic testing for Pendred syndrome and *SLC26A4*-related HL.[39,145,146] However, many nongenetic tests have low diagnostic yield in patients with HL.[147] Furthermore, recent advances in genetic testing technologies that permit the analysis of many genes simultaneously at rapidly decreasing cost may soon prompt reassessment of the clinical utility of certain nongenetic tests as part of the initial workup for the etiologic diagnosis of HL. Such reassessments will need to consider the clinical utility of various nongenetic tests vs the risks associated with those tests, such as the clinical utility of computed tomography and magnetic resonance imaging vs the risks associated with radiation exposure and sedation.[17,147] As evidence for the clinical utility of NGS tests for the etiologic diagnosis of HL is accumulated and evaluated, physicians should continue to rely on their best clinical judgment and consider the use of nongenetic tests for the evaluation of HL on a case-by-case basis. For example, unless cochlear implantation is being considered, auditory neuropathy is detected, progressive HL is identified, or other specific clinical concerns exist, it could be argued that temporal bone imaging might, in some cases, be better used as a complement or follow-up to genetic testing rather than as a part of the initial diagnostic evaluation.[147,148] For example, it might be used to aid in the evaluation of a VUS detected in the *SLC26A4* gene associated with Pendred syndrome. In addition, in the absence of specific clinical concerns or family history, tests such as electrocardiographic studies, thyroid function testing, urinalysis, and renal ultrasonography might also be postponed until results of genetic testing are obtained and then ordered as clinically indicated.[147,149,150]

Recommendations

1. All newborns and infants with confirmed HL should undergo a comprehensive evaluation in which patient-focused medical and birth histories, a 3-generation pedigree, and family medical history are obtained, and a physical examination that focuses on dysmorphic physical findings is performed. Evaluation of children and young adults with HL should follow a similar approach. Evaluation of deaf or hard-of-hearing adults should be customized based on the age of onset and other characteristics of HL (Figure 1).
   - The medical and birth histories may be helpful in differentiating between acquired vs inherited causes of HL. Elements of medical and birth histories focused on HL include the following:
     - Prenatal history, including maternal infections (eg, CMV, rubella) and illnesses (eg, syphilis), or medication or drug exposures (eg, thalidomide, retinoic acid)[151,152]
     - Neonatal history, including premature birth, low birth weight, birth hypoxia, hyperbilirubinemia, sepsis, and exposure to ototoxic medications
     - Postnatal history, including viral illnesses, bacterial meningitis, head trauma, noise exposure, and exposure to ototoxic medications. (Accessed February 4, 2022. https://www.ninds.nih.gov/Disorders/Patient-Caregiver-Education/Fact-Sheets/Meningitis-and-Encephalitis-Fact-Sheet).

- Audiometric assessment of HL, including sensorineural vs conductive or mixed HL; age of onset; progressive, nonprogressive, or fluctuating nature of HL; laterality, symmetry, severity, and configuration of HL; and presence or absence of vestibular dysfunction or auditory neuropathy

- The pedigree and family medical history should focus on identifying the following:
  - First- and second-degree relatives with HL or with features commonly associated with HL (such as pigmentary, branchial, retinal, or renal anomalies) or sudden cardiac death
  - A pattern of inheritance
  - Ethnicity and country of origin
  - A common origin from ethnically or geographically isolated areas
  - Consanguinity
- The physical examination should focus on dysmorphic and other physical findings such as the following:
  - Unusual facial appearance, with attention to asymmetry
  - Pigmentary anomalies
  - Neck, skin, facial, or ear anomalies
  - Neurological abnormalities
  - Balance disturbances
  - Skeletal abnormalities
  - Other unusual physical findings

2. For individuals with findings that suggest a syndromic genetic etiology for their HL:
   - Pretest genetic counseling should be provided, and, with patient's or caregiver's informed consent, genetic testing should be ordered to confirm the diagnosis. This testing may include single-gene tests, HL multigene panels, ES, GS, chromosome analysis, or microarray- or NGS-based copy number analysis, depending on clinical findings;
   - Appropriate studies should be undertaken to determine whether other organs are involved; and
   - Appropriate near-term and long-term screening and management should be arranged, including referrals to specialists, as indicated by the associated manifestations of the particular syndrome.
3. For individuals lacking physical findings suggestive of a known syndrome, a tiered diagnostic approach should be implemented.
   - Unless clinical and/or family history suggests a specific genetic etiology, comprehensive HL gene panel testing should be initiated. If panel testing is negative, genome-wide testing, such as ES or GS, may be considered. However, issues related to genomic testing, such as the likelihood of incidental or secondary findings, will have to be addressed.
     - The HL panel should include the genes recommended by the HL Gene Curation Expert Panel (https://clinicalgenome.org/affiliation/40007/#heading_documents).[131] Because of the existing variations in gene number and content among currently available HL gene panels, clinicians must be aware of the genes included in the test (panel) chosen and the performance characteristics of the platform chosen, including coverage, analytic sensitivity, and what types of variants will be detected. Additional testing strategies may need to be adopted to address the technical challenges caused by highly homologous regions, including pseudogenes. It should be noted that the cost of these new genetic sequencing technologies is decreasing so rapidly that the use of large sequencing panels targeted toward HL-related genes as the initial test, may, in many cases, already be more cost-effective in the evaluation of HL.
     - If genetic testing reveals variant(s) in an HL-related gene, gene-specific genetic counseling should be provided, followed by appropriate medical evaluations and referrals.
     - If genetic testing fails to identify an etiology for a patient's HL, the possibility of a genetic etiology remains. This point must be emphasized because it can be misunderstood by clinicians and by patients and their families. For interested patients and families, further genetic testing may be pursued on a research basis.
   - Temporal bone imaging by computed tomography or magnetic resonance imaging should be considered as a complement to genetic testing, particularly if the diagnosis remains unclear; if cochlear implantation is being considered; if auditory neuropathy is noted, in cases of progressive HL; or if other clinical concerns exist. The anticipated clinical utility of imaging studies should be balanced against the risks associated with radiation exposure and sedation.
   - CMV testing should be done as soon as possible after birth but within the first 3 weeks of life for infants with congenital HL. For later-onset or progressive HL, CMV testing can be obtained, but the likelihood that a positive test is caused by postnatal exposure increases with age.
4. Referral to a multidisciplinary care center, when available, is recommended.
   - A team approach that includes otolaryngologists, clinical geneticists, genetic counselors, audiologists, speech and language specialists, early hearing intervention and family support specialists (which may include other individuals who are deaf or hard of hearing or other parents of deaf or

hard-of-hearing children), and other appropriate specialists offers optimal opportunity to provide ongoing management and support of deaf and hard-of-hearing individuals and their families as their needs change over time.

- For cases in which the genetic evaluation failed to identify an underlying cause, periodic follow-up care every 3 years with a geneticist may be appropriate for several reasons. First, subtle features of syndromic forms of HL may not be apparent at birth or early in childhood but may appear as deaf or hard-of-hearing individuals grow into adulthood. These may prompt additional medical tests or referrals for specialty care. Second, follow-up visits offer the opportunity to inform individuals about new genetic tests that may have become available or changes in the interpretation of previous test results as medical knowledge advances. Finally, follow-up visits may also help identify clinical concerns unrelated to HL, for which referral for specialty care may be appropriate (Figure 1).

5. Regardless of whether genetic test results are positive, negative, or inconclusive, results should be communicated through the process of genetic counseling and potential risks to other family members should be conveyed.

Future Perspectives

Early detection of HL in newborns is critical for intervention and promoting language development. Although the current physiologic NBHS has significantly improved outcomes of newborns with HL, it may miss mild congenital HL, later-onset childhood HL, risk factors for aminoglycoside-induced HL, and auditory neuropathy, resulting in potentially preventable adverse outcomes. A proposal of integrating universal genetic screening and cCMV screening into the current NBHS to improve detection and early intervention of newborns with HL was published in 2019 by the Newborn Hearing Screening Working Group of the National Coordinating Center for the Regional Genetics Networks.[18] A few preclinical trials are underway to evaluate the efficacy of different gene therapy strategies for HL early intervention, further emphasizing the importance of early etiologic diagnosis.[153,154] Currently, the considerable cost of sequencing and complexity of result interpretation are the major hurdles for universal genetic screening. As sequencing costs decrease and the knowledge regarding the genes and variants associated with all childhood diseases, including HL, improves, genetic screening is likely to become part of more comprehensive universal newborn screening in the near future. This will certainly result in early audiologic and etiologic detection of HL with its many benefits to be realized.[155-157]

Conflict of Interest

M.M.L., A.A.T., M.D., and H.L.R. serve as directors in not-for-profit clinical laboratories that offer genetic and genomic testing for hearing loss. M.M.L. is on the Scientific Advisory Board for Bayer HealthCare Pharmaceuticals Inc. A.M.S. works with the Molecular Otolaryngology and Renal Research Laboratories (MORL), a not-for-profit clinical genetic testing laboratory that provides genetic testing for hearing loss. The remaining authors declare no conflict of interest.

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