Next-gen diagnostics and newborn screening for hearing loss

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Abstract

Universal newborn hearing screening has been implemented since the 1990s following technological developments of routine automated screening methods to test a baby’s hearing. In countries with successful newborn screening programs, the age at diagnosis of hearing loss has been reduced dramatically, and early intervention to mitigate adverse consequences of hearing loss realized. However, despite the tremendous value of the traditional screening methods, there are limitations, including high false positive and negative rates, difficulty in distinguishing transient versus permanent hearing loss, inability to predict and prevent hearing loss presymptomatically, and lack of an etiologic diagnosis. In this era of precision medicine, etiologic diagnoses are fundamental to facilitate individualized management and targeted therapeutics. Given the majority of congenital hearing loss is hereditary with unparalleled genetic heterogeneity, next-generation sequencing and computing methods for rapid discovery of the genetic basis of a hearing loss are technological developments that will mark another milestone in newborn hearing screening. Implementation of next-gen newborn hearing screening will result in improved sensitivity and specificity, will enable precision diagnosis at an early age, and has potential to reduce healthcare costs and societal disparities.
Introduction

Hearing loss is the most common sensory deficit in humans leading to speech and language delays, challenges in school, work and relationships, and isolation and depression in the elderly. For babies, a critical time for habilitation is during the first few months of life. This paper addresses several questions. Firstly, why is genetic testing so important for hearing impairments? What is the landscape of genetic testing for hearing loss? What is the current status of universal newborn screening? And, lastly, what is the future of testing and screening for hearing disorders?

Background

It is important for us to consider hearing loss across the lifespan. Concerning newborns in the United States (U.S.), approximately two to three of every 1,000 children are born with permanent hearing loss, making it one of the most common birth defects in the U.S. The majority of children with hearing loss are born into families with little or no experience with hearing loss. Furthermore, greater than 90% of deaf by deaf matings result in hearing offspring, highlighting unparalleled heterogeneity in the etiology of deafness—including genetic, environmental, and gene X environmental effects. Approximately 50% of children with hearing loss are from racial or ethnic minority populations ("Regional and National Summary Report of Data from the 2002-2003 Annual Survey of Deaf and Hard of Hearing Children and Youth," 2003). About one in two cases of hearing loss in babies are due to genetic causes, while about one in four cases is due to a maternal infection during pregnancy, complications after birth, and/or head trauma (Centers for Disease Control and Prevention, 2015). By school age, hearing loss is present in approximately one in 100 children (Shargorodsky, Curhan, Curhan, & Eavey, 2010; White, Forsman, Eichwald, & Munoz, 2010), accounting for about 2.5 million (or 5.4%) of all school-age children with mild or unilateral hearing loss (ASHA, 2008).

Turning to age-related hearing impairment (ARHI), about one in three individuals over age 65 years have hearing loss significant enough to impair speech perception. By the year 2030, over 20% of the U.S. population will be greater than 76 years old (U.S. Census Bureau). ARHI is more prevalent in males than in females. It is a complex trait with genetic and environmental factors contributing to onset and progression. To date, there has been little progress in understanding the underlying molecular basis. However, recent genetic methods empowered by big data resources from the human genome project, such as genome wide association studies (GWAS), provide an approach to deciphering complex traits and are being applied to ARHI research.

Hearing loss is categorized into various types, including whether it is unilateral or bilateral and whether it is conductive (affecting the outer ear or middle ear) or sensorineural (affecting the inner ear). It can be mild, moderate or profound, stable or progressive, or part of a syndrome or isolated and known as non-syndromic. The categorization or phenotypic description of hearing loss can be important in trying to develop an understanding of the relationship of the underlying genetic etiology to the phenotype.

The causes of hearing loss are very heterogeneous and, in fact, have been categorized by the term unparalleled heterogeneity—they can be largely broken down into environmental and genetic etiologies. Environmental origins of hearing loss include ototoxic drugs, noise trauma, infection, and malnutrition. Genetic causes can be sub-categorized into syndromic etiologies representing about 30% of cases, and into nonsyndromic etiologies in about 70% of cases. Syndromic cases include disorders such as Alport, Branchial–Oto–Renal (BOR), CHARGE, Jervell and Lange-Nielsen, Norrie, Pendred, Perrault, Stickler, Treacher-Collins, Usher, Waardenburg, and Wolfram syndromes. Among the nonsyndromic disorders are those with autosomal dominant (DFNA, 22%), autosomal recessive (DFNB, 77%), X-linked (DFNX, 1%), Y-linked (DFNY), and mitochondrial inheritance.

As of 2015, about 123 genes for hearing loss had been identified; about 90 of those 123 represent nonsyndromic hearing loss disorders, 62 represent nonsyndromic recessive disorders, and 30 autosomal dominant nonsyndromic; 43 represent syndromic disorders in which hearing loss is a major clinical feature, including some associated with both dominant and recessive inheritance and with both nonsyndromic and syndromic forms. This represents remarkable gene discovery progress in hearing loss since 1990, at which time the National Institute of Deafness and Other Communication Disorders (NIDCD) had its first budget to fund research in this area.

Genetic testing for hearing loss

It is important to consider the clinical utility of genetic testing and its value across the lifespan. Newborn hearing screening can lead to early intervention for hearing impairments. Genetic testing can facilitate precision management and also contribute to valuable information for family planning and risk assessment. Genetic information can lead to prenatal testing in subsequent pregnancies as well as important information for lifelong management. As an ever increasing number of genetic discoveries for hearing loss genes are made, gene test menus increase. Questions have...
been asked about who gets to choose whether testing will be offered and which type of testing will be performed (Harmon, 2004).

A hearing loss screening protocol can be designed based around the finding of nonsyndromic and syndromic causes of hearing loss. An important nonsyndromic cause of hearing loss is cytomegalovirus. A nonsyndromic etiology could be best addressed by initial GJB2/GJB6 testing due to its frequency and then a tiered choice of gene tests based on family medical history. A history of maternal transmission in conjunction with the finding of a history of aminoglycoside treatment would lead to a possible mitochondrial etiology with testing for the 12S rRNA and tRNA serine genes. An X-linked familial segregation and characteristic temporal bone findings could lead to prioritized testing for POU3F4. Nearly 100 genes for hearing loss are now recognized for both dominant and recessive patterns of inheritance, and the absence of any affected family members can be suggestive of a recessive form of deafness or de novo occurrence of a dominant pathogenic variant. In the setting of a syndromic form of hearing loss, prioritizing for testing based on unique clinical features can limit testing to a specific gene (SLC26A4 or WFS1 for Pendred or Wolfram syndromes, respectively) or a collection of genes recognized for involvement in a disorder with overlapping clinical findings such as for Usher (ADGRV1, CDH23, CIB2, CLRN1, DFNB31, HARS, MYO7A, PCHD15, USH1C, USH1G, USH2A), Waardenburg (EDN3, EDNRB, MITF, PAX3, SNAI1/2, SOX10), or Perrault (CLPP, HARS2, HSD17B4, LARS2) syndromes.

A recent analysis considered gene panel inclusion criteria for nonsyndromic or apparently nonsyndromic hearing loss genes (Abou Tayoun et al., 2016). Beginning with 163 hearing loss genes, a triage approach was initiated first by assessing whether the hearing loss gene was likely to be syndromic, followed by an assessment as to whether the hearing loss was the presenting feature. If the hearing loss was not likely to be syndromic and evaluation of the evidence level of the gene disease association was considered next, and in conjunction with those potentially syndromic cases in which an evidence level of 2 or 3 (likely or strong disease association, respectively) was identified, 90 (55%) genes were selected for the panel and 73 (45%) genes excluded.

The Laboratory for Molecular Medicine (LMM) at Partners Personalized Medicine (http://personalizedmedicine.partners.org/Laboratory–For-Molecular–Medicine) has developed a next-generation sequencing panel known as OtoGenome that includes 87 genes. An analysis of 959 cases assessed on the OtoGenome panel resulted in 23% of cases receiving a conclusive diagnosis with a dominant pathogenic or likely pathogenic allele or with biallelic recessive inheritance, and 7% represented pathogenic variants in genes for Usher syndrome; in most cases GJB2-related hearing loss had been excluded prior to OtoGenome testing. The majority of cases (59%) were diagnosed as inconclusive, with about 20% having a single recessive allele considered to be pathogenic and the remaining having a variant considered to be of uncertain clinical significance. Ten percent of cases were reported as negative because only benign or likely benign variants were identified. Among 218 OtoGenome cases with a positive diagnosis, the most common etiologies were STRC (23.7%), GJB2 (22.7%), and SLC26A4 (8.1%) in addition to genes for Usher syndrome, indicating that the majority of cases are caused by a handful of genes in this series of patients. Because GJB2 testing is the first tier test, and patients who are positive for GJB2 will not undertake the OtoGenome test, GJB2 is still the most common cause of hereditary deafness. Furthermore, considering hearing loss variants in over 3,000 cases tested at the LMM, 817 variants were classified as pathogenic, likely pathogenic, or of unknown clinical significance, and 673 (82.4%) variants were only detected in a single family. These data indicate that to achieve a higher detection rate the testing strategy must include a comprehensive panel that targets a large number of genes and that can also detect all types of variants. It should be noted that an assessment of copy number variants is necessary to rule out deletions in some genes for hearing loss such as those more frequently identified in USH2A and STRC.

A large number of panels for hearing loss using next-generation sequencing tests are now available around the world. A comparison of 14 of these panels reveals the number of genes tested to be as high as about 250 in Hong Kong to 52 genes on a syndromic panel in Brazil with diagnostic yields ranging from 10 to 72%. A variety of capture techniques including TruSeq, SureSelect, AmpliSeq and NimbleGen were employed and sequencing was performed using Illumina, Ion PGM, and Ion Torrent platforms. Explanations for the variable diagnostic yields include inclusion or exclusion of GJB2-tested patients, stringency of variant classification, and interpretation, small sample sizes in some reported studies, family history (i.e., simplex vs. multiplex), specific clinical features, and ethnicity (i.e., consanguinity, availability of population data). In an analysis of 1,119 patients with hearing loss who underwent comprehensive genetic testing by OtoSCOPE in their clinical evaluation, the underlying genetic cause for hearing loss was determined in 440 patients (39%). The diagnostic rate varied considerably as assessed by the following variables: inheritance, age of onset, severity, laterality, physical exam and previous testing. The solve rate was highest for patients with a positive family
history of hearing loss or when the hearing loss was congenital and symmetric (Sloan-Heggen et al., 2016).

**Newborn hearing screening**

Newborn hearing screening is the standard of care in the U.S. with greater than 98% of newborns screened prior to leaving the birth hospital. Two common screening methods are employed: otoacoustic emissions (OAEs) and auditory brainstem response (ABR), detecting hearing loss in the frequency region important for speech recognition. Approximately 1.6% are documented not to have passed final hearing screening, and about 45% of those not passing newborn hearing screening are lost to follow-up or documentation for diagnosis. The prevalence of documented hearing loss is approximately 1.4% per 1,000 newborn screened. Of those with hearing loss, there are documented referrals of about 88% to Early Hearing Detection and Intervention (EDHI) programs. Approximately 50% of states have language in legislation or regulations that includes coverage for early intervention services for children with mild or unilateral hearing loss.

On October 28, 1988 Public Law 100 – 553 authorized the formation of the NIDCD and established its mission areas to include research on hearing, balance, taste, and smell, and voice, speech and language. In March 1993, universal newborn hearing screening was recommended by the NIH for the early detection of hearing loss because undetected hearing loss has serious negative consequences for language acquisition. Dramatic benefits are associated with the early identification of hearing loss and intervention is recommended at less than six months of age. The Centers for Disease Control and Prevention (CDC, www.cdc.gov) has noted that approximately four infants are born every day with bilateral hearing impairments that will not be diagnosed in time to prevent significant lifelong communication disabilities, and without early detection programs the current average age at which children are identified as having hearing impairment was 30 months. Even more astounding figures are reported by the National Center for Hearing Assessment and Management at Utah State University (NCHAM, www.usu.edu/~ncham/index). NCHAM found that 33 babies are born each day in the U.S. with permanent hearing loss representing 12,000 annually, and with three of every 1,000 births having a hearing loss, it is the most frequently occurring birth defect. The average age of identification of hearing impairment in the U.S. was 31 months prior to universal newborn hearing screening, compared to an average of 6 to 7 months in countries such as England and Israel.

Next-gen newborn screening for hearing can set the paradigm for expanded genetic screening. Why? It makes a difference for treatment and management to know the precise diagnosis – the foundation of precision medicine. Because hereditary hearing loss displays unparalleled genetic heterogeneity it offers an opportunity to simulate a complex genetic disorder in the context of hundreds of single gene defects.

Precise diagnosis impacts care. It is already recognized that individuals with GJB2 deafness benefit from cochlear implantation. In contrast, individuals with biallelic pathogenic variants in DFNB59 (encoding peyvakin) can be harmed from amplification. Identification of the genetic etiology of hearing loss can lead to other groups with potential for optimized habilitation. Furthermore, some syndromic disorders can be indistinguishable from nonsyndromic disorders at birth such as Alport, BOR, Jervell and Lange-Nielsen, Pendred, and Usher syndromes.

Many conversations have occurred about the need for an etiologic diagnosis for newborn hearing screening (Morton & Nance, 2006) and the optimal time for genetic testing to take place (Schimmenti et al., 2004), including at birth, following failed audiometry from newborn screening, and following confirmed hearing impairment. Positive reasons for genetic testing at birth include testing of all newborns, the potential for a reduced medical workup, the possibility to use blood spots, and the opportunity to identify those at risk. Negative factors include false positives, the detection of many carriers and potential for low parental interest. Positive aspects of genetic screening following failed audiometry from newborn screening include a reduction in the number of infants tested with overlap of a reduced workup and possibility for use of blood spots. Akin to the negative factors at birth are false positives, detection of many carriers and potential for low parental interest; in addition testing at this time point can miss some at risk and would in some cases likely require new specimens. Genetic testing following confirmed hearing impairment would result in a further reduction in infants being tested and with a reduced work up, and would permit specific testing with likely high parental interest. As with testing following failed audiometry from newborn screening, some infants at risk would likely be missed, a new specimen would be required, and in contrast to the previous time periods it would result in a delay in intervention.

Over the past few years with the development of next-generation sequencing methods and various resources made available through the human genome project, discussions matured around the possibility of implementing genomic screening into newborn screening. In 2012, a request for applications from NIH to implement genomic testing into newborn screening (RFA-HD-13-010) accelerated the interest. Implementation of genomic sequencing into newborn
screening for hearing loss could serve as a valuable precedent for furthering this effort. A comparison of various aspects of traditional hearing screening and next-generation newborn hearing screening make clear some advantages (Shen & Morton, 2016) beyond an etiologic diagnosis and its implications for accurate recurrence risk estimation, possibility for improved prognosis and optimal management, and for advanced therapeutics through precision medicine. Sensitivity for the potential to detect hearing loss with a later onset is expected to increase and specificity for detection of transient hearing loss categorized as a false positive is anticipated to improve. A pilot study of implementing whole genome sequencing into newborn hearing screening is being launched at the Brigham and Women’s Hospital, known as SEQaBOO (Sequencing a Baby for an Optimal Outcome), with potential to identify viral sequences such as cytomegalovirus. SEQaBOO will inform three important clinical aspects of genomic hearing screening of newborns and infants: general acceptance in clinical practice, clinical validity, and clinical utility. This study is anticipated to provide important new insights into strategies for the management of childhood hearing loss and also improve the lives of families and the roles of clinicians who care for them.

Conclusion

In summary, universal programs to screen newborns for hearing defects throughout the world has truly been a revolution in health care, but would benefit greatly from the introduction of an etiologic focus, and the improved identification of infants at risk for later-onset hearing loss. Genomic testing on all newborns sets the stage for the future of precision medicine for all, and a proof of the application might be forthcoming from its deployment in newborn hearing screening.

References


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