Mini Review

Genomic advances for gene discovery in hereditary hearing loss

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Abstract

High-throughput sequencing is changing the face of genetic diagnosis and counseling. While in the past, it would take on average 1 to 5 years to identify a mutation leading to deafness, today, the genetic basis for deafness can be determined within months in a child or adult with inherited hearing loss. Obstacles and challenges still remain, but the field is changing at a dramatic rate, making gene discovery a much easier and more efficient task than in the past.

Keywords: consanguineous; deafness; homozygosity mapping; massively parallel sequencing; next-generation sequencing.

The genetics basis of hearing loss

A majority of hearing loss is inherited (1). As a consequence, a large effort has been made for many years to discern all the genes involved in hereditary hearing loss. This information is not only crucial for patients wanting to know the cause of their hearing loss, but it has also provided a great deal of data about the mechanisms and pathways of hearing loss and auditory function (2). Studies on patients worldwide have provided the elements required to find these genes. In particular, consanguineous families in regions of the world, including the Middle East and India and Pakistan, have facilitated gene discovery. Hereditary hearing loss is subdivided into modes of inheritance (1). Autosomal dominant (AD) hearing loss, with a tendency to be postlingual and progressive in nature, may be part of a syndrome or occur alone. Examples of the former include Waardenburg, Stickler, and Treacher Collins syndromes. AD non-syndromic hearing (NSHL) loss tends to be less common, accounting for approximately 18% of all hereditary deafness, although it is more prevalent in certain parts of Europe (e.g., Belgium). Autosomal recessive (AR) hearing loss tends to have a prelingual onset [although there are exceptions, e.g., DFNB30 with MYO3A mutations (3)]. Syndromic forms are Usher, Pendred, and Jervell and Lange-Nielsen syndromes. ARNSHL is the most prevalent form worldwide, particularly in regions of consanguinity. Less abundant forms of NSHL are X-linked and mitochondrial inheritance, although the latter is quite prevalent in Spain (4).

The syndromic forms of hearing loss have been labeled with letters representing the syndrome, e.g., USH for Usher syndrome, followed by a number, defining the order in which the subtype of the disease was found (e.g., USH1A-F, 2A-D, USH3). For NSHL, the different forms have been defined by DFNA for dominant and DFNB for recessive, followed by a number, defining the order in which the locus was discovered (Hereditary Hearing Loss homepage; http://hereditaryhearingloss.org/).

Since the chromosomal mapping of the first ADNSHL locus for DFNA1 in 1992 (5) and for ARNSHL for DFNB1 in 1994 (6), a large number of genes have been identified containing mutations shown to lead to hearing loss (7). This reflects the genetic heterogeneity of hearing loss, as almost 160 different forms of NSHL have been found to date (Hereditary Hearing Loss homepage). What is particularly captivating is the variety of genes and proteins they encode, as well as the types of mutations leading to hereditary hearing loss. Most compelling, studies of patients from geographical regions with high rates of ARNSHL have contributed a significant number of genes.

Homozygosity mapping to facilitate gene discovery

There are two main advantages to studying recessive diseases in highly inbred populations, and hearing loss has been no exception. Inbreeding reveals recessive mutations and increases the prevalence of recessive disease. Within an inbred population, the number of affected individuals is proportional to the disease allele frequency, whereas in a large, randomly mating population, the number of affected is proportional to the square of the disease allele frequency. Thus, inbreeding increases the number of available patients for study. A second
reason is that genetic mapping in consanguineous families reduces genetic heterogeneity. The underlying assumption is that when a recessive disease arises in a consanguineous family, it must be due to a mutation that is identical by descent, and affected children are expected to be homozygous by descent for the disease haplotype, i.e., the causative mutation and closely linked genetic markers. All unaffected siblings will not be homozygous for the disease haplotype, and within the family, obligate carriers (i.e., parents of affected children) will all carry only one copy of this same disease haplotype. Homozygosity mapping is particularly powerful because a disease locus can be identified even in families too small to obtain the “classic” threshold of a high (>3) LOD score (8).

How have genes been identified to date? The majority have been by identification of the genetic locus containing the mutant gene by linkage analysis, followed by Sanger sequencing to identify the mutation causing the disease (9). In families where a novel locus was identified, positional cloning strategies were used to identify the causative gene. Genes were prioritized for positional cloning on the basis of factors such as the size of the candidate region (the smaller the common homozygous region, the higher the priority) and the existence of relevant candidate genes with the minimal homozygous region. Once a region was identified, all known and predicted genes within the minimal homozygous regions were found through the University of California, Santa Cruz Genome Browser (http://genome.ucsc.edu/) and other online resources. Each gene was examined for genomic structure, regulatory regions, and alternative splice forms to ensure that all exons were sequenced. Candidate genes for sequencing were prioritized on the basis of available online and experimental resources, including disease associations in humans, phenotypes in mice with mutations/knockouts of the homologous genes, known tissue expression patterns, sequence, and predicted domain homology to genes associated with related diseases. Publicly available bioinformatics tools for prioritizing candidate genes within a genomic region have also been used (10), incorporating gene annotations, protein-protein interactions, regulatory information, gene expression data, sequence information, and text mining.

Many, if not most, of the genes for deafness have been discovered using the above strategy. These include connexin 26, or GJB2, as the gene is named, now known to be responsible for the most common form of ARNSHL DFNB1 (11). This locus was first identified in consanguineous families from Tunisia. Mutations in connexin 26 were subsequently found (11, 12). Connexin 26, along with other connexins, form connexons that form gap junctions, facilitating transfer of small molecules between cells and across membranes (13). An essential component in the inner ear, impaired connexin 26 gap junctions in the inner ear are thought to have defective K+ recycling, ultimately leading to deafness. To this date, many genetics clinics only examine patient’s DNA for connexin 26 mutations, owing to its prevalence and ease in diagnostics.

The ensuing years have added >100 genes to the area of molecular basis of deafness (7). Despite this success, the methods for examining mutations in a large number of genes simultaneously have not been optimal. Primer extension has been reported as a useful tool to screen for 198 mutations in eight prevalent genes (14). Affymetrix resequencing microarrays have been used clinically to resequence 13 genes containing mutations for hearing loss (15). However, these techniques have been at a significant cost to the patient, and limited to only a portion of mutations tested. In other cases, mutations in specific genes have been examined on the basis of previously found mutations in the same ethnic groups. These include the more common mutations found in genes such as SLC26A4, TMCI, MYO7A, and TMPRSS3.

Deep sequencing: the new generation of gene identification

Deep sequencing, also referred to as next-generation sequencing or massively parallel sequencing, is changing the face of genetic diagnosis in human disease (9, 16), deafness notwithstanding. For the first time, the ability to screen for mutations in hundreds of genes is possible. Deep sequencing, as opposed to the conventional capillary sequencing, allows sequencing at a high depth of coverage so that each base pair is read hundreds of times. The major portions of deep sequencing include template preparation, sequencing and imaging, and data analysis, with a variety of commercial platforms available (17, 18). The amount of genomic sequence that can be sequenced has increased phenomenally. Whereas the first human genome sequence, of J. Craig Venter, by the Sanger method cost an estimated $70,000,000, Stephen Quake’s cost $48,000 and the costs today are much lower and decreasing toward $1000 (17).

The first generation of such deep sequencing experiments involved targeted capture and exome sequencing of a chromosomal region, following linkage analysis that had previously determined the chromosomal region to study. An example is the identification of the tazerin (TPRN) gene, encoded by the DFNB79 locus, in an extended Palestinian family with prelingual and profound ARNSHL (19). Another option has been to deep sequence the entire exome, but focus the bioinformatics analysis to search for a mutation only in homozygous regions, previously determined by homozygosity mapping. Such an example is the discovery of the GPSM2 mutation in an extended Palestinian family with prelingual and profound deafness (20). Other studies captured a defined number of genes previously known to be involved in deafness, followed by deep sequencing of the exons of these genes only (21, 22). The majority of deafness genes, both syndromic and non-syndromic, found thus far by deep sequencing has involved either targeted capture of a specific region based on previous linkage, or capture of a limited number of genes [reviewed in ref. (23)].

The second round of such experiments involves capture of the entire exome on several unrelated individuals with the same phenotype. This was done for AD cerebellar ataxia, deafness and narcolepsy (ADCA-DN), a late-onset form of cerebellar ataxia, narcolepsy-cataplexy, and dementia with deafness (24). Exome sequencing was performed in five affected members from three unrelated pedigrees. As bioinformatics tools become more efficient for identifying mutations
in large genomic regions and the costs of deep sequencing come down, exome sequencing will become more prevalent. A schematic diagram describing the overall strategy for exome sequencing to identify a disease gene, using homozygosity mapping, is shown in Figure 1.

Ultimately, whether these genomic advances will change the treatment of hereditary hearing loss remains to be seen. Genomic advances have been making a difference in some diseases; however, given the high cost, the work is mostly on a research, and not clinical diagnostic, level. This is sure to
change very soon. A number of other diseases have benefitted from deep sequencing. When siblings with a rare movement disorder, Segawa dystonia, had their genomes sequenced, pathogenic mutations found in the sepiapterin reductase gene dictated the course of treatment (26). Exome sequencing, which led to the discovery of a mutation in the X-linked inhibitor of apoptosis gene, defined the treatment of allogeneic hematopoietic progenitor cell transplant for a child with a Crohn disease-like illness (27).

The future is bright for finding all, or most, of the causative mutations for hereditary hearing loss, using the latest genomic technologies that continue to develop at a remarkable pace. The challenges that remain are primarily in how the mechanisms of deafness will be defined and how therapeutics may be used to treat or cure this sensory disease, using genetic or other means (28, 29).

**Acknowledgments**

Research in the Avraham and Kanaan laboratories for human genomics research is funded by the National Institutes of Health (National Institute on Deafness and Other Communication Disorders) R01DC011835. We thank Daphne Karfunkel for creating Figure 1.

**Conflict of interest statement**

**Authors’ conflict of interest disclosure:** The authors stated that there are no conflicts of interest regarding the publication of this article. Research funding played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

**Research funding:** None declared.

**Employment or leadership:** None declared.

**Honorarium:** None declared.

**References**


