Genetics, genomics and gene discovery in the auditory system

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The sounds of silence have forever been broken as genetics and genomics approaches in human and model organisms have provided a powerful and rapid entry into gene discovery in the auditory system. An understanding of the complexities and beauty of the biological process of hearing itself is unfolding as genes underlying hereditary hearing impairment are identified. Genes involved in modifying hearing are also being found, and will be critical to a full comprehension of genotype–phenotype relationships. Investigations in the auditory system will provide important insight into how the nervous system decodes molecular information. Deafness represents a common sensory disorder that can interfere dramatically in the acquisition of speech and language in children, and in the quality of life for a growing aged population. As newborn screening for hearing impairment is being implemented in many birth hospitals, the prospects for precise clinical diagnosis, appropriate genetic counseling and proper medical management for auditory disorders has never been at a more exciting crossroad.

INTRODUCTION

It has long been recognized that heredity plays a major role in hearing impairment. Despite the fact that understanding the genetic basis of hearing loss has fascinated human and medical geneticists for decades, only within the past few years have the genes and molecular mechanisms underlying deafness begun to be discovered. In part, this results from various obstacles to investigation of the auditory system including: inaccessibility of the sensory end organ for hearing, the cochlea, within the dense temporal bone; length of time to direct pathologic observation of the deaf ear due to an otherwise normal lifespan of individuals with hearing loss; unparalleled genetic heterogeneity; and assortative mating. The history of the genetics of deafness has had a sordid past of blatant discrimination of the deaf (1), and there is mistrust about genetics among some members of the deaf population. Nevertheless, the past few years have witnessed an explosion of discoveries that are providing fundamental insight into the biology of hearing.

The frequency of hearing loss is estimated at one per thousand newborns and half of all cases are attributed to genetic causes. In addition to being a common etiology of congenital deafness, mutations in genes are responsible for progressive hearing loss, and no doubt will be found to play an important role in progressive hearing loss with ageing (presbycusis). Environmental etiologies of hearing loss are likely to represent a declining proportion of cases as better therapies for bacterial and viral infections (e.g. vaccines) are implemented, acoustic trauma in the workplace is recognized and prevented, and ototoxic drugs (e.g. aminoglycosides) are avoided in genetically susceptible individuals.

GENETICS OF DEAFNESS

The study of the genetics of deafness is unique among inherited disorders for several reasons and illustrates well various concepts in human genetics. Notably, there is incomparable genetic heterogeneity with over 90% of matings among the deaf resulting in all hearing offspring. This reflects matings among the deaf with mutations in different genes as well as matings of couples in which one individual is deaf due to a genetic mechanism and the other due to an environmental etiology. Matings among the deaf are well recognized and, with the exception of assortative mating for stature, may represent one of the most common genetic traits on which an altered mating structure occurs in human populations. Furthermore, the hearing offspring of deaf couples who themselves can be native signers are more likely than random members of the population to have a partner who is deaf due to a shared language and culture. Both genetic heterogeneity and assortative mating confound gene discovery using traditional methods of genetic linkage analysis.

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<td>Usher syndrome type 1F (USH1F)</td>
<td>(111)</td>
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Hundreds of syndromic forms of deafness have been described (2-4) and the underlying genetic mutation identified for many of the more common forms, but only 30% of genetic cases are estimated to be part of a heritable syndrome. Thus, the vast majority of genetic deafness is designated as nonsyndromic and to date over 65 loci have been mapped. Nonsyndromic hearing impairment is categorized further by mode of inheritance: approximately 77% of cases are autosomal recessive; 22% autosomal dominant; 1% X-linked; and < 1% due to mitochondrial inheritance (5). Dominant loci are designated with the prefix ‘DFN’; recessive loci ‘DFNB’, X-linked loci ‘DFN’ and modifying loci with ‘DFNM’. Generally, patients with autosomal recessive hearing impairment have prelinguistic and congenital deafness and patients with autosomal dominant impairment have postlinguistic and progressive hearing impairment. This observation may be explained by the complete absence of functional protein in recessive disorders, while in autosomal dominant disorders, dominant mutations may be consistent with initial function and subsequent hearing impairment due to a cumulative, degenerative process. A recent tally of nonsyndromic hearing loss disorders reveals 32 autosomal dominant, 27 autosomal recessive, and 4 X-linked forms (4). It remains to be shown whether each of these loci will correspond to a unique gene. In fact, various deafness disorders have been found already to be the result of the same genetic etiology (e.g. DFNA8 and A12; DFNA6, A14 and A38). In addition, at least 58 auditory genes have been identified: 16 for autosomal dominant and 11 for autosomal recessive loci, and 1 for an X-linked locus; 6 mitochondrial genes and at least 38 genes for syndromic hearing loss have also been discovered (n.b. some genes cause multiple forms of deafness) (Table 1). Although this magnitude of progress is remarkable and significant advances have been made, it is clear that many more genes for hearing await detection.

Mutations within the same gene have been found to result in a variety of clinical phenotypes with different modes of inheritance. For example, mutations in MYO7A are patho- genetic in the autosomal recessive deafness and blindness disorder Usher syndrome type 1B (USH1B), and in two nonsyndromic hearing disorders, DFNB2 and DFNA11, displaying autosomal recessive and dominant segregation, respectively. It has been suggested that the mutation in DFNA11, a 9 bp deletion in the coiled-coil domain of MYO7A which is involved in dimerization, could have a dominant negative effect (6) as compared to splicing and missense mutations observed in recessive USH1B and DFNB2 (7-9). Another example of phenotypic heterogeneity also involving Usher syndrome is seen in mutations in CDH23 detected in USH1D and DFNB12 (10,11). Mutations in PDS cause Pendred syndrome and nonsyndromic autosomal recessive deafness.

### Table 1. (Continued)

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<td>NSHL, DFNA15</td>
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<td>PMP22</td>
<td>ribosomal RNA</td>
<td>SHL, Charcot-Marie-Tooth disease</td>
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<td>12S RNA</td>
<td>mitochondrial</td>
<td>NSHL, Sensorineural deafness</td>
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<td>TECTA</td>
<td>alpha tectorin</td>
<td>NSHL, Thiamine-responsive megaloblastic anemia with diabetes mellitus and deafness</td>
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<td>SLC26A4</td>
<td>pendrin</td>
<td>NSHL, SHL, PDS</td>
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<td>SOX9</td>
<td>treacle</td>
<td>SHL, Diabetes and deafness</td>
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<td>stereocilin</td>
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hearing loss, DFNB4. Similarly, mutations in WFS1 cause Wolfram syndrome and also account for an autosomal dominant nonsyndromic deafness, DFNA6/A14/A38. Most recently, DFNA36 and DFNB7/B11 were determined to be the result of mutations in TMC1 (12).

**GENOMIC APPROACHES TO GENE DISCOVERY IN THE AUDITORY SYSTEM**

Traditional methods for mapping disease genes, such as genetic linkage analysis, have a less than totally optimal use in gene discovery efforts for hearing disorders, mainly because of the complex genetic nature of deafness. Successful use of genetic linkage for mapping hearing disorders, especially for autosomal recessive nonsyndromic loci, has been restricted largely to consanguineous kindreds or populations in which there has been limited immigration. Even in families in which a heritable hearing disorder is successfully mapped, there is often an insufficient number of recombination events to narrow a chromosomal interval, resulting in a candidate region consisting of megabases of genomic DNA. Positional cloning has been productive for a modest number of human deafness genes including NDP (13,14), TCOF1 (15), DDP (16), SLC26A4 (17), USH2A (18) and DFNA5 (19). Positional candidate genes from human (e.g. COL4A5 (20), TECTA (21), COCH (22), COL4A4 and 4A4 (23), GJB2 (24,25), GJB3 (26)) and mouse (e.g. Pax3 (27), Mitf (28), Otomericyelia (29), Ush1C (30), Strc (31)) among others have been the primary method for gene identification.

Organ and tissue-specific methods for auditory candidate genes

A complementary method to genetic linkage analysis for gene identification is one that utilizes tissue or organ-specific cDNA libraries to provide candidate genes (32–34). A transcript map of the inner ear provides a ready source of positional candidate genes for mutation screens in gene discovery efforts. To this end, cochlear cDNA libraries constructed from human (35,36) and mouse (37) have provided precious biological tools for gene discovery in the mammalian cochlea. A cDNA library from an analogous organ in chicken, the basilar papillae, has also been of great value (38). Almost 15 000 human (Morton fetal cochlear cDNA library) and 1600 mouse (Soares mouse NMIE cDNA library) inner ear ESTs are currently available in GenBank and the sequences of an additional 9000 mouse ESTs will soon be deposited there. ESTs derived from two sequencing projects from human cochlear cDNA clones (39,40) have elucidated thousands of potential positional candidate genes for hearing disorders (41) in addition to providing a snapshot of gene expression in the 16–22 week gestational age fetal cochlea. BLAST analysis of 8153 human ESTs revealed that about 50% (n=4040) had sequence similarity to a total of 1449 known human genes. The most abundantly expressed gene was COL1A2, and two other collagens, COL3A1 and COL1A1, were among the most highly represented transcripts. In total, 10 different collagen genes were present in the cochlear ESTs. Forty-three human homologs of nonhuman mammalian genes were also identified, and among them are ESTs for membrane proteins, extracellular proteins and trafficking proteins. Of the remaining 4055 ESTs,
Ames waltzer (av) & Pcdh15 & Usher syndrome type 1F (USH1F) & (103,104,128)  
Beethoven (Bth) and deafness (dn) & Tmc1 & DFNA 36, DFNB7/B11 & (12,50)  
Chondrodyplasia (cho) & Coll1a1 & Stickler syndrome type 2 (STL2) & (81,129,130)  
Coll1a2 targeted null & Coll1a1 & Osteogenesis imperfecta (OI) & (131–134)  
Coll4a3 targeted null & Coll1a2 & Stickler syndrome type 3 (STL3), DFNA13 & (82,83)  
Disproportionate micromelia (Dmm) & Coll4a3 & Alport syndrome & (23,135)  
and mutant transgenes & Coll2a1 & Stickler syndrome type 1 (STL1) & (80,136,137)  
Dominant megacolon (Dom) & Sox10 & Waardenburg-Shah syndrome (WS4) & (111,138,139)  
dominant spotting (W) & Kit & Piebald trait (PBt) & (140–142)  
Eya1^{Vld} and targeted null & Eya1 & Branchio-oto-renal syndrome (BOR) & (88,143,144)  
Fgf3 targeted null & Fgf3 & Craniosynostosis & (145,146)  
Gata targeted null & Gata3 & Hypoparathyroidism, sensorineural deafness & (147,148)  
and renal dysplasia syndrome (HDR) &  &  &  
Kcnel targeted null and Kcnel^sh & Kcnel & Jervell and Lange-Nielsen syndrome (JLNS2) & (91,92,149,150)  
Kcnq1 targeted null & Kcnq1 & Jervell and Lange-Nielsen syndrome (JLNS1) & (94,151,152)  
lateral spotting (ls) & Edn3 & Waardenburg-Shah syndrome (WS4) & (86,153)  
microphthalmia (mi) & Mlf & Waardenburg syndrome type 2 (WS2), & (28,95,154,155)  
Ndp targeted null & Ndp & Norrie disease (ND1) & (13,14,156)  
Pax2 targeted null & Pax2 & Renal-coloboma syndrome & (157,158)  
piebald (s) & EdnrB & Waardenburg-Shah syndrome (WS4) & (87,159)  
Pou3f4 targeted null and sex-linked fidget (slf) & Pou3f4 & DFN3 & (105, 160–162)  
Pou4f3 targeted null and dreidel (ddl) & Pou4f3 & DFNA15 & (69,163,164)  
quivering (qv) & Spnb4 & Charcot-Marie-Tooth disease type 4F (CMT4F) & (165)  
shaker-1 (sh1) & Myo7a & Usher syndrome type 1B (USH1B), DFNB2, & (7,98,166,167)  
&  & DFNA11, atypical Usher syndrome &  
shaker-2 (sh2) & Myo15a & DFNB3 & (99,168)  
Slc26a4 targeted null & Slc26a4 & Pendred syndrome (PDS), DFNB4 & (17,109,169)  
Snell's waltzer (sv) & Myo6 & DFNA22 & (48,49)  
plochut (Sp) & Pax3 & Waardenburg syndromes types 1 and 3 (WS1, WS3), & (27,101,102,170,171)  
&  & Craniofacial dysmorphism, hand abnormalities, profound sensorineural deafness (CDHS) &  
Tecta targeted null & Tecta & DFNA8A12, DFNB21 & (21,112,172)  
Thrd targeted null & ThrB & Thyroid hormone resistance & (173,174)  
Tremble (Tr) & Pmp22 & Charcot-Marie-Tooth disease type 1A (CMT1A) & (106,175,176)  
Waltzer (v) & Cdh23 & Usher syndrome type 1D (USH1D), DFNB12 & (10,11,177)  

In some instances, identification of the mouse mutation has greatly preceded detection of the human disorder (48,49),
whereas in other cases discovery of the genetic basis for deafness has occurred concurrently (12,50). Positional cloning of deafness genes in the mouse is facilitated by the ability to breed large numbers of mice with the same mutation to narrow the interval for study. A large screen of inbred mouse strains by ABR threshold analyses at The Jackson Laboratory is currently underway, and likely to identify mutants that will lead to discovery of new genes and modifying genes for deafness (51). In addition, large numbers of new mouse mutants for investigating mammalian gene function including deafness are being generated rapidly through ENU mutagenesis (52). This chemical mutagenesis program also makes possible gene-driven approaches to mouse mutants and using this approach, two missense and one stop mutation were recently identified in Gjb2, the most common cause of nonsyndromic deafness in the human population (53). Besides the spontaneous deaf mouse mutants and those generated from mutagenesis programs, a number of gene targeting experiments have been performed following identification of the human gene, and have provided valuable mouse mutants for investigation. As the human and mouse DNA sequencing projects are finished, the mouse-human synteny maps will also become better defined and it will become increasingly easier to locate potential mouse mutants for mapped human deafness disorders.

**GENE DISCOVERY IN THE AUDITORY SYSTEM: THE PATH TO IMPROVED DIAGNOSIS AND CLINICAL CARE**

Inspection of the genes identified in hearing disorders and those among the gene lists from the EST sequencing projects reveals a great diversity of transcripts, perhaps not surprising due to the large variety of cells and complexity of the inner ear. The finding of a large percentage of cochlear ESTs not identified in any other tissue may indicate the existence of genes that are unique to the cochlea. Certainly, the great degree of genetic heterogeneity reflected in the many different syndromes involving hearing loss and mapped loci is indicative of a large number of genes orchestrating the hearing process. Grouping the genes discovered to be etiologic in deafness disorders into functional categories begins the process of understanding their role in hearing. Knowledge of the pathways in which many of these genes function will be an exciting journey in hearing science; no doubt pathways exist that are not yet imagined. Another important aspect of gene discovery for deafness disorders is that it makes possible the development of diagnostic tests and accurate genetic counseling. Appropriate medical management and therapeutic options may be based on an understanding of the specific disorder.

**Gap junction proteins: the connexins**

Prominent among the group of genes are those encoding gap junctions. A somewhat surprising finding in the field has been the prevalence of mutations in a single gene encoding the gap-junction protein connexin 26, Gjb2, accounting for up to 50% of all cases of autosomal recessive prelingual deafness in tested populations (54–65). Connexin 26 gap junctions are believed to play a critical role in the recycling of potassium ions from their entry into hair cells during sensory transduction from the endolymph through to the stria vascularis, where other potassium channels pump potassium back into the endolymph. The gap junction itself, the connexon, is formed from six monomers of connexin and forms a pore between cells by binding with a connexon on an adjacent cell. Several recurrent mutations have been found in Gjb2 (e.g. 35delG, 167delT, and 235delC), some with ethnic predilections. In addition to the recurrent mutations, the gene is small (681 bp) making it especially amenable for genetic testing. Screening for mutations in Gjb2 has already emerged as the cornerstone of genetic testing for hearing loss and has been incorporated in some centers into the clinical work-up of infants who fail newborn hearing tests. The connexin-deafness homepage provides information on connexin mutations in deafness (Table 2).

Besides Gjb2, genes for other gap junction proteins have been found to be associated with hearing loss including Gjb3 (26), Gjb6 (66) and Gja1 (67). A curious finding in genetic testing of the deaf for Gjb2 has been the frequent observation of heterozygosity for a mutation. Various explanations have been proposed including the possibility that the deafness was due to another gene or that there was a mutation in a non-coding region of Gjb2 not evaluated in the test. Recent identification of a 342 kb deletion in the Gjb6 gene (Δ(Gjb6-D13S1830)) as the second most frequent mutation causing prelingual deafness in the Spanish population may provide the sought after answer in many cases (68). Gjb6 maps within the same chromosomal region as Gjb2, and these recently published data suggest that mutations in the DFNB1 locus can result in a monogenic or digenic pattern of inheritance. Of note, the typical mutation-detection assays commonly in use may miss such large deletions.

**Genes for maintenance of hair cell function**

Another group of genes of intense interest are those required for survival of sensory hair cells. The POU domain transcription factor gene Pou4f3 is required for terminal differentiation and maintenance of inner hair cells and an 8 bp deletion in the POU homeodomain results in progressive hearing loss in DFNA15 (69). Studies of such genes may result in valuable insight into the molecular triggers for hair cell degeneration. Loss of hair cells is presumed to be a fundamental cause of progressive age-related hearing loss (presbycusis) and an understanding of this degenerative process might provide the basis for therapeutic intervention. The recent finding of the transmembrane cochlear-expressed gene Tmc1 uncovers a common cause of nonsyndromic recessive deafness in Pakistan and India at the DFNB7/B11 locus on chromosome 9 in bands q13–q21; mutations in Tmc1 account for the deafness phenotype in 5.4±3.0% of 230 families screened (12). Cloning of Tmc1 resulted from an interesting genomics-based approach that first involved identification of a predicted gene (subsequently designated Tmc2) on chromosome 20 with sequence similarity to query sequences in a TBLASTx analysis of a BAC from the linked genetic interval. Tmc1 mutations were also found to be etiologic in DFNA36 (12) and in the mouse mutants deafness (dn) (12) and Beethoven (Bth) (50). It is predicted that Tmc1 protein may mediate an ion-transport or channel function required for the normal function of hair cells.
The recessive dn mutant displays no auditory response and has secondary hair cell degeneration and the dominant 8th mutant appears to have normally functioning hair cells prior to degeneration.

**Modifier genes**

Molecular analyses of the auditory system have already yielded a number of genes in mice and humans that influence the expression or function of other genes. Studies of these genes are certain to provide insight into the interaction of their gene products. Notable among the mouse genes are the expression or function of other genes. Studies of these modifier genes and mouse models continue to yield new and novel genes providing valuable insight into the molecular basis of the process of hearing.

REFERENCES

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