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CHAPTER 2

GENETICS IN OTOLOGY AND NEUROTOLOGY

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SEARCH STRATEGY

Data in this chapter may be updated by a Medline search using a variety of relevant generic keywords, including: DNA, genetics, hereditary hearing loss, connexin 26, neurofibromatosis 2 and familial paragangliomas.

INTRODUCTION

Advances in molecular genetics have helped to identify causative genes and proteins responsible for pathologies; this knowledge is pertinent to molecular target therapy and promises novel therapeutic interventions.

This chapter aims to review the mechanisms and principles of molecular genetics of hearing impairment, vestibular schwannoma, and glomus tumours, and will keep the reader abreast of new developments that may be relevant to identifying and treating these diseases in clinical practice.

MOLECULAR GENETICS OF HEARING LOSS

Hearing loss is the most frequent sensory impairment in humans, with significant social and psychological implications. Permanent childhood hearing impairment of a moderate or greater degree (i.e. detection thresholds 40 decibels hearing level averaged across 0.5, 1, 2 and 4 kHz) is present at birth in about 1.6 per 1000 live births, of which approximately 1.0 in 1000 are bilateral impairments and 0.6 in 1000 are unilateral impairments.[1,2] However, studies have shown that the prevalence of permanent childhood hearing impairment continues to increase through infancy, and by the school entry hearing screen (4–5 years of age) possibly affects 3.5 per 1000 children.[2] It is estimated that at least two thirds of cases of childhood-onset hearing loss have a genetic cause, with the remaining third caused by environmental factors (e.g. cytomegalovirus infection, meningitis, acquired conductive loss, and the impact of extracorporeal membrane oxygenation (ECMO)).[3] Improved clinical knowledge, awareness, and advances in antibiotics and vaccines has led to a decline in hearing loss resulting from environmental factors. Likewise, significant progress in the genetics of hereditary hearing impairment (HHI) has improved our understanding of the causes and early detection of HHI.

Classification

The most useful distinction in hereditary hearing impairment (HHI) is syndromic versus non-syndromic. When SNHL occurs in isolation it is termed non-syndromic and when it is accompanied by other systemic disturbances it is termed syndromic. The majority of hearing impairments are non-syndromic (~70%), whereas a minority are syndromic (~30%).

Non-syndromic HHI is further classified by mode of inheritance:

- Autosomal recessive ~80%
- Autosomal dominant ~18%.[4]

Rare modes of transmission include X-linked and mitochondrial transmission, which account for the remaining 2% of hearing impairment.

MOLECULAR GENETICS OF NON-SYNDROMIC HEARING IMPAIRMENT (NSHI)

Thus far, more than 100 forms of non-syndromic hearing impairment (NSHI) have been discovered (see the Hereditary Hearing Loss website at http://hereditaryhearingloss.org). Most of these are still identified as loci: only the chromosomal location of the defective gene is known.

The different gene loci for non-syndromic deafness are designated DFN (for DeaFNess). Loci are named based on mode of inheritance:

- DFNA: Autosomal dominant
- DFNB: Autosomal recessive
- DFNX: X-linked.

The number following the above designations reflects the order of gene mapping and/or discovery and this information is regularly updated on the Hereditary Hearing Loss website.

Genes for NSHI have been categorized by the function of the proteins each gene encodes. Several superfamilies are represented, including gap junctions, myosins, adhesion proteins and ion channels.

Unlike in some conditions where a common mutation in just one gene is responsible for the majority of cases (e.g. the deltaF508 deletion of three nucleotides in the *CFTR* gene in cystic fibrosis), hearing loss is genetically very heterogeneous involving mutations in many genes.[5] It is therefore not possible to predict the risk of developing hearing loss from assessing a single or even a selection of the genes that have currently been identified.[6]

The most frequent causative genes are discussed below.

Gap junction proteins

Thus far, three connexin genes have been implicated in NSHI: *CX26*, *CX30*, and *CX31*, corresponding to the proteins connexin 26, connexin 30, and connexin 31, which are designated by their molecular mass.

GJB2 (connexin 26) is by far the foremost prominent hearing impairment gene. The discovery that *GJB2*, located at the DFNB1 locus, is a causative gene for hearing impairment[7] led to the unexpected revelation that more than half of the recessive NSHL and approximately 30% to 50% of innate hearing loss are due to *GJB2* mutations.[7,8] Among the 300 mutations documented worldwide up to now (see The Human Gene Mutation Database at www.hgmd.org), one particular mutation, 35delG, is responsible for 70% of all the *GJB2* mutations.[9] This finding made an enormous impact in the hearing loss field, as it facilitated diagnostic and genetic screening of NSHL patients. Another mutation imperative to note is 167delT, which is most frequent among Ashkenazi Jews and is also present in Palestinians.[10]

The connexion-deafness homepage (http://davinci.crg.es/deafness/) provides a database of all published mutations as well as common polymorphisms in *GJB2* and other connexin genes. More than 100 unique non-syndromic *GJB2* gene variants have already been described. The reported mutations mostly include mutation types that can be detected by sequencing analysis, such as nonsense, missense, splicing and frameshift mutations.

Myosins

Numerous types of unconventional myosins have been identified as NSHI causing genes: *MYO1A*, *MYO3A*, *MYO6*, *MYO7A* and *MYO15A* (encoding myosin I, myosin IIIA, myosin VI, myosin VIIA and myosin XVA proteins, respectively). The term 'unconventional' myosins refers to all non-muscle myosin subclasses (in contrast with the 'conventional' myosins that include only the myosin II subclass). Expression of these unconventional myosins is not limited to the cells and tissues of the inner ear, yet the expression of their dysfunction is largely restricted to hearing impairment.

The myosin VI protein was first implicated in the hearing mechanism when it was found to be mutated in the spontaneously deaf mouse Snell's *waltzer* (*sv*).[11] The role of myosin VI in human deafness remains to be determined.

Myosin VII has been linked to both rodent and human deafness. Mutations in the *MYO7A* human homologue were identified as the genetic cause for Usher syndrome type 1B[12] and Usher syndrome type 2A.[13] Contiguous to this discovery, human *MYO7A* was also linked to NSHL in two distinctive loci: DFNB2[14] and DFNA11.[15]

Myosin XV is the largest of all the myosin heavy chains and mutations in myosin XV have been pathogenically linked to DFNB3 in humans and shaker 2 phenotype in mice. Myosin XV is a critical factor in the evolvement of the hair bundle and in the staircase formation of the bundle[16] and it binds another deafness molecule, whirling at the tip link zone, suggesting that the two molecules might control stereocilia elongation patterning during development and may be implicated in stabilizing connections between stereocilia.[17]

Pendrin

Mutations in the pendrin gene *SLC26A4* are the second most frequent cause of autosomal recessive NSHI, accounting for up to 3.5% of cases.[18] *SLC26A4*, which codes for an anion transport protein, also underlies Pendred syndrome, which is one of the most common forms of syndromic hearing impairment.

Adhesion proteins

Adhesion proteins include a diverse group of distinct protein families including integrins, selectins, members of the immunoglobulin superclass family and cadherins.

Cadherin 23 is a vital member of a specific unit designated for maintaining stereocilia inter-cohesion.[18]

More than 20 mutations in the *CDH23* gene have been identified in people with non-syndromic hearing impairment and more than 30 mutations have been shown to cause the Usher syndrome type 1D. The *USH1D* locus was found to coincide with the recessive NSHI locus *DFNB12*, which is associated with prelingual moderate to profound hearing loss. These data suggested that *DFNB12* and *USH1D* in fact are mutually derived from *CDH23* allelic mutations, both resulting in syndromic and non-syndromic forms of hearing loss.[19, 20]

Autosomal dominant non-syndromic hearing impairment

Unlike autosomal recessive non-syndromic hearing loss, in autosomal dominant non-syndromic hearing loss there is not a single identifiable gene responsible for the majority of cases. The clinical manifestations and molecular genetics of known genes causing autosomal dominant non-syndromic hearing impairment are outlined in Table 2.1.

Autosomal recessive non-syndromic hearing impairment

Fifty per cent of people with autosomal recessive non-syndromic hearing impairment have mutations in *GJB2*.[8] The other 50% of cases are attributed to mutations in numerous other genes. The clinical manifestations and molecular genetics of known genes causing autosomal recessive non-syndromic hearing impairment are outlined in Table 2.2.

X-linked non-syndromic hearing impairment

DFNX3 is characterized by a mixed conductive-sensorineural hearing loss, the conductive component of

TABLE 2.1 Clinical manifestations and molecular genetics of known genes causing autosomal dominant non-syndromic hearing impairment

Locus name	Gene symbol	Onset/Decade	Audioprofile	Test availability
DFNA1	*DIAPH1*	Postlingual/1st	Low frequency progressive	Clinical
DFNA2	*KCNQ4*	Postlingual/2nd	High frequency progressive	Clinical
DFNA2B	*GJB3*	Postlingual/4th	High frequency progressive	Clinical
DFNA3	*GJB2*	Prelingual	High frequency progressive	Clinical
	GJB6			Clinical
DFNA4	*MYH14*	Postlingual	Flat/gently downsloping	Clinical
DFNA5	*DFNA5*	Postlingual/1st	High frequency progressive	Clinical
DFNA6/14/38	*WFS1*	Prelingual	Low frequency progressive	Clinical
DFNA8/12	*TECTA*		Mid-frequency loss	Clinical
DFNA9	*COCH*	Postlingual/2nd	High frequency progressive	Clinical
DFNA10	*EYA4*	Postlingual/3rd, 4th	Flat/gently downsloping	Clinical
DFNA11	*MYO7A*	Postlingual/1st		Clinical
DFNA13	*COL11A2*	Postlingual/2nd	Mid-frequency loss	Clinical
DFNA15	*POU4F3*	Postlingual	High frequency progressive	Clinical
DFNA17	*MYH9*	Postlingual	High frequency progressive	Clinical
DFNA20/26	*ACTG1*	Postlingual	High frequency progressive	Clinical
DFNA22	*MYO6*	Postlingual	High frequency progressive	Clinical
DFNA23	*SIX1*	Prelingual	Downsloping	Clinical
DFNA25	*SLC17A8*	Postlingual/2nd–6th decades	High frequency progressive	Clinical
DFNA28	*TFCP2L3*	Postlingual	Flat/gently downsloping	Clinical
DFNA36	*TMC1*	Postlingual	Flat/gently downsloping	Clinical
DFNA39	*DSPP*	Postlingual	High frequency progressive	Research only
DFNA44	*CCDC50*	Postlingual	Low to mild frequencies progressive	Clinical
DFNA48	*MYO1A*	Postlingual	Progressive	Clinical
DFNA50	*MIR96*	Postlingual/2nd	Flat progressive	Clinical
DFNA51	*TJP2 & FAM189A2*	Postlingual/4th	High frequency progressive	Research only

Source: Adapted from Van Camp & Smith [2010] Van Camp G, Smith RJH. The Hereditary Hearing Loss Homepage, 2010, with permission. Available from http://hereditaryhearingloss.org/.

TABLE 2.2 Clinical manifestations and molecular genetics of known genes causing autosomal recessive non-syndromic hearing impairment

Locus name	Gene symbol	Onset	Type	Test availability
DFNB1	*GJB2*	Prelingual	Usually stable	Clinical
	GJB6			Clinical
DFNB2	*MYO7A*	Prelingual, postlingual	Unspecified	Clinical
DFNB3	*MYO15*	Prelingual	Severe to profound; stable	Clinical
DFNB4	*SLC26A4*	Prelingual, postlingual	Stable, progressive	Clinical
DFNB6	*TMIE*	Prelingual	Severe to profound; stable	Clinical
DFNB7/11	*TMC1*			Clinical
DFNB8/10	*TMPRSS3*	Postlingual, prelingual	Progressive, stable	Clinical
DFNB9	*OTOF*	Prelingual	Usually severe to profound; stable	Clinical
DFNB12	*CDH23*	Prelingual	Severe to profound; stable	Clinical
DFNB16	*STRC*	Prelingual	Severe to profound; stable	Clinical
DFNB18	*USH1C*	Prelingual	Severe to profound; stable	Clinical
DFNB21	*TECTA*	Prelingual	Severe to profound; stable	Clinical
DFNB22	*OTOA*	Prelingual	Severe to profound; stable	Clinical
DFNB23	*PCDH15*	Prelingual	Severe to profound; stable	Clinical
DFNB24	*RDX*	Prelingual	Severe to profound; stable	Clinical
DFNB25	*GRXCR1*	Prelingual	Moderate to profound; progressive	Research only
DFNB28	*TRIOBP*	Prelingual	Severe to profound; stable	Clinical
DFNB29	*CLDN14*	Prelingual	Severe to profound; stable	Clinical
DFNB30	*MYO3A*	Prelingual	Severe to profound; stable	Clinical
DFNB31	*DFNB31*	Prelingual	–	Clinical
DFNB32/82	*GPSM2*	Prelingual	Severe to profound; stable	Research only
DFNB35	*ESRRB*	Unknown	Severe to profound	Clinical
DFNB36	*ESPN*	Prelingual	–	Clinical
DFNB37	*MYO6*	Prelingual	–	Clinical
DFNB39	*HGF*	Prelingual	Severe to profound; downsloping	Clinical
DFNB49	*MARVELD2*	Prelingual	Moderate to profound; stable	Clinical
DFNB53	*COL11A2*	Prelingual	Severe to profound; stable	Research only
DFNB59	*PJVK*	Prelingual	Severe to profound; stable	Clinical
DFNB61	*SLC26A5*	Prelingual	Severe to profound; stable	Clinical
DFNB63	*LRTOMT*	Prelingual	Severe to profound; stable	Clinical
DFNB67	*LHFPL5*	Prelingual	Severe to profound; stable	Clinical
DFNB73	*BSND*	Prelingual	Severe to profound; stable	Research only
DFNB77	*LOXHD1*	Postlingual	Moderate to profound; progressive	Clinical
DFNB79	*TPRN*	Prelingual	Severe to profound; stable	Clinical
DFNB84	*PTPRQ*	Prelingual	Moderate to profound; progressive	Research only

Source: Adapted from Van Camp & Smith [2010] Van Camp G, Smith RJH. The Hereditary Hearing Loss Homepage, 2010, with permission. Available from http://hereditaryhearingloss.org/.

which is caused by stapedial fixation. In contrast to other types of conductive hearing loss, surgical correction is precluded because an abnormal communication between the cerebrospinal fluid and perilymph results in leakage ('perilymphatic gusher') and complete loss of hearing when the oval window is fenestrated or removed.

Clinical manifestations and molecular genetics of known genes causing X-linked non-syndromic hearing impairment are summarized in Table 2.3.

Mitochondrial non-syndromic hearing impairment

The genes and mutations associated with mitochondrial non-syndromic hearing impairment are outlined in Table 2.4.

TABLE 2.3 Clinical manifestations and molecular genetics of X-linked non-syndromic hearing impairment

Locus name	Gene	Onset	Type and degree	Frequencies
DFNX1 (DFN2)	*PRPS1*	Postlingual	Progressive sensorineural; severe to profound	All
DFNX2 (DFN3)	*POU3F4*	Prelingual	Progressive, mixed; variable, but progresses to profound	All
DFNX4 (DFN6)	*SMPX*	Postlingual	Progressive sensorineural; mild to profound	All

Source: Adapted from Van Camp & Smith [2010] Van Camp G, Smith RJH. The Hereditary Hearing Loss Homepage, 2010, with permission. Available from http://hereditaryhearingloss.org/.

TABLE 2.4 Mitochondrial Non-syndromic Hearing Impairment

Gene symbol	Mutation	Severity	Penetrance	Test availability
MT-RNR1	961 different mutations	Variable	Highly variable, aminoglycoside induced	Clinical
	1494C>T			
	1555A>G			
MT-TS1	7445A>G		Highly variable	Clinical
	7472insC			
	7510T>C			
	7511T			
MT-CO1	7444G>A	Severe to profound	Complete, aminoglycoside associated; associated with *MT-RNR1* 1555A>G	Clinical

Source: Adapted from Van Camp & Smith [2010] Van Camp G, Smith RJH. The Hereditary Hearing Loss Homepage, 2010, with permission. Available from http://hereditaryhearingloss.org/.

Molecular genetics of syndromic hearing impairment (SHI)

Hearing impairment is denoted as an integral clinical phenotype in more than 400 genetic syndromes.[21] Syndromic forms of hearing impairment are estimated to be responsible for up to 30% of prelingual deafness, but its contribution to deafness is relatively small reflecting the occurrence and diagnosis of postlingual deafness.

Syndromic hearing loss is discussed further by modes of inheritance.

Autosomal dominant syndromic hearing impairment

WAARDENBURG SYNDROME (WS)

WS is mostly a genetic autosomal dominant disorder, considered to be the most frequent autosomal dominant form of syndromic hearing loss, constituting approximately 2% of all congenital hearing impairment.[22]

The primary phenotypes observed in this syndrome may include variable degrees of sensorineural hearing loss, pigmentation abnormalities (skin, hair, eyes), dystopia canthorum (wide distance between the inner corners of the eyes). Gastrointestinal symptoms like constipation and Hirschsprung disease, neural tube and limb defects may also be observed. Four different types of WS (I to IV) have been described, grouped by distinct physical characteristics. WS I characteristically has the presence and WS II the absence of dystopia canthorum. In WS III there are typically limb abnormalities and in WS IV Hirschsprung disease is present.

All of WS1 and some of WS3 cases are associated with mutations in the *PAX3* gene. WS2 is caused by mutations in the transcription factors *MITF* and *SNAI2*. WS4 is due to alterations in *EDNRB*, *EDN3* or *SOX10* genes.[23]

BRANCHIOOTORENAL SYNDROME

Branchiootorenal (BOR) syndrome is the second most prevalent autosomal dominant syndromic type of hearing loss.[24] The major features of this syndrome are hearing loss (conductive, mixed or sensorineural) in association with branchial cleft cysts or fistulae, deformities of the external, middle or internal ear, and renal malformations.

In approximately 40% of families segregating a BOR phenotype, mutations in *EYA1* can be identified; in a few other families mutations have been found in *SIX1*[25] and *SIX5*.[26]

STICKLER SYNDROME

Stickler syndrome comprises of progressive sensorineural hearing loss, cleft palate, abnormal development of the epiphysis, vertebral abnormalities and osteoarthritis. Three types are recognized, based on the molecular genetic defect: STL1 *(COL2A1)*, STL2 *(COL11A1)* and STL3 *(COL11A2)*. STL1 and STL2 are characterized by severe myopia, which predisposes to retinal detachment; this aspect of the phenotype is absent in STL3 because *COL11A2* is not expressed in the eye.