NON-SYNDROMIC HEARING LOSS AND HIGH-THROUGHPUT STRATEGIES TO DECIPHER ITS GENETIC HETEROGENEITY

Liu Xue Zhong¹, Shan Kun², Qing Jing¹, Cheng Jing³, YanDenise¹

Abstract
Hearing loss (HL) is the most common sensory disorder, affecting all age groups, ethnicities, and genders. According to World Health Organization (WHO) estimates in 2005, 278 million people worldwide have moderate to profound HL in both ears. Results of the 2002 National Health Interview Survey indicate that nearly 31 million of all non-institutionalized adults (aged 18 and over) in the United States have trouble hearing. Epidemiological studies have estimated that approximately 50% of profound HL can be attributed to genetic causes. With over 60 genes implicated in nonsyndromic hearing loss, it is also an extremely heterogeneous trait. Recent progress in identifying genes responsible for hearing loss enables otolaryngologists and other clinicians to apply molecular diagnosis by genetic testing. The advent of the $1000 genome has the potential to revolutionize the identification of genes and their mutations underlying genetic disorders. This is especially true for extremely heterogeneous Mendelian conditions such as deafness, where the mutation, and indeed the gene, may be private. The recent technological advances in target-enrichment methods and next generation sequencing offer a unique opportunity to break through the barriers of limitations imposed by gene arrays. These approaches now allow for the complete analysis of all known deafness-causing genes and will result in a new wave of discoveries of the remaining genes for Mendelian disorders. This review focuses on describing genotype-phenotype correlations of the most frequent genes including GJB2, which is responsible for more than half of cases, followed by other common genes and on discussing the impact of genomic advances for comprehensive genetic testing and gene discovery in hereditary hearing loss.

Introduction
Hearing impairment is the most common sensory disorder worldwide. It affects approximately 3% of the population. When present from birth it can affect a child’s development, not only linguistically but also in ambulation and the formation of social relationships. In cases of postlingual deafness, affected individuals often suffer from social isolation and the inability to adapt to a noisy environment.

Many etiologies have been identified for hearing impairment, both environmental and genetic. Environmental causes can range from neonatal insults, such as prematurity, jaundice, or prenatal infection and iatrogenic causes. The genetic etiologies can be inherited as either syndromic or nonsyndromic forms, and have a spectrum of inheritance patterns. Currently, genetics may account for approximately 50% of all childhood deafness. Recent research has focused on identifying the genetic causes that may be important not only to the patient but also in assessing the success of certain therapies such as cochlear implants.

In the past, establishing a genetic etiology based on the clinical presentation has only been possible for syndromic deafness, whereby patients present with classic audioligic and systemic manifestations. However, a
great challenge exists in assessing a genetic cause for patients who present with nonsyndromic hearing loss. Classically, all that may be untangled at the time of presentation of the inheritance pattern of the deafness. For example, autosomal recessive deafness typically presents as a congenital or prelingual, severe to profound deafness affecting all frequencies while autosomal dominant deafness is more often postlingual with variations in severity and affected frequencies. There are of course exceptions that make particular genetic causes easier to identify. This review highlights phenotypes associated with nonsyndromic deafness in an effort to help evaluation of patients and to establish a role for genetic testing in some of these patients. The impact of genomic advances for comprehensive genetic testing and gene discovery in hereditary hearing loss is also discussed.

**Epidemiology of Non-Syndromic Loss**

Hearing loss affects approximately 70 million people worldwide. 50-60% of these cases have a genetic etiology; the remaining 40-50% of cases are attributed to environmental factors, such as ototoxic drugs, prematurity, or trauma. However, as public health awareness is improved, environmental factors are contributing less to the etiology of deafness and the relative proportion of genetic hearing loss is increasing\[66\]. Approximately, one in every 1000 children has some form of prelingual hearing impairment\[74\], and 1 in 2000 is caused by a genetic mutation. 30% of cases of prelingual deafness are classified as syndromic; the remainders are non-syndromic.

The inheritance of nonsyndromic deafness depends upon the time of onset. Prelingual types have a predominantly (80%) autosomal recessive inheritance, although 20% are inherited in an autosomal dominant pattern, 1% are X-linked, and less than 1% are mitochondrial inheritance\[66\]. Postlingual types are most commonly autosomal dominant, whereas autosomal recessive forms are rare but more severe.

Non-syndromic deafness is a paradigm of genetic heterogeneity. To date, 69 genes have been identified (24 for dominant and 40 for recessive deafness; Hereditary Hearing Loss Homepage, June, 2013) with new genes being discovered at a rapid pace. This review summarizes the most relevant information about different genes associated with nonsyndromic deafness along with their proposed molecular function in the auditory system, and describes the phenotypes that have been correlated to specific genotypes.

Non-syndromic loci are being discovered at an unbelievable pace. Currently, 145 loci have been discovered—57 for autosomal dominant, 77 autosomal recessive, 8 X-linked, 2 modifier and 1 Y linked deafness. Some of these loci share a common gene, while others have unknown genes. Nonetheless, each type of inheritance has a generalized clinical picture that applies to most of the genes or loci. Autosomal recessive deafness tend to cause severe, prelingual deafness involving all frequencies; autosomal dominant loci are typically less severe, postlingual, with hearing loss that can vary by frequency; lastly, X-linked loci affect males more severely than females and can impair all frequencies or only high fre-

<table>
<thead>
<tr>
<th>OMIM Number</th>
<th>Locus</th>
<th>Gene (MIM)</th>
<th>Location</th>
<th>Gene</th>
<th>Protein</th>
<th>Predicted Function</th>
<th>Age of Onset</th>
<th>Affected frequency</th>
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<td>DFNA1</td>
<td>602121</td>
<td>5p31</td>
<td>DIAPH1</td>
<td>Diaphanous Homolog 1 (Drosophila)</td>
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<td>0–20</td>
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<td>Wolframin</td>
<td>Integral, endoglycosidase</td>
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Table 1. Cloned Nonsyndromic Deafness Genes and Their Phenotypes
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<tr>
<th>Genes</th>
<th>Chromosome</th>
<th>Genomic Location</th>
<th>Gene Product</th>
<th>Genes</th>
<th>Chromosome</th>
<th>Genomic Location</th>
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<td>Myosin VIIA</td>
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<td>All or low frequency</td>
<td>Symptoms of Vestibular dysfunction</td>
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<td>Collagen 11A2</td>
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<td>Transcription factor</td>
<td>20–40 (with variability to puberty)</td>
<td>All frequency</td>
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<td>13q11–q12</td>
<td>GJB2</td>
<td>connexin 26</td>
<td>Gap junction protein</td>
<td>prelingual</td>
<td>All frequency</td>
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<td>GJB6</td>
<td>connexin 30</td>
<td>Gap junction protein</td>
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<td>All frequency</td>
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<td>Otoferlin</td>
<td>Synaptic vesicle component</td>
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<td>DFNB22</td>
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<td>OTOA</td>
<td>Otoancorin</td>
<td>Anchoring protein between acellular gels and non–sensory cells</td>
<td>prelingual</td>
<td>All frequency</td>
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Genotype-Phenotype Corelations of Common Forms of Non-Syndromic Hearing Loss

Most gene discoveries are based on a single family where deafness has been inherited. While nonsyndromic genes only affect hearing, their expression is not necessarily limited to the inner ear. It may be that the inner ear is simply more sensitive to the identified mutation.

Genes can be classified by their molecular function. This review will classify the genes by the following functions: Homeostasis, Hair Cell Structure, Transcription Factors, Cytokinesis, Extracellular Matrix, Mitochondrial and Other/Unknown.

**Cochlear Ionic Homeostasis**

Homeostasis is maintained by a number of mechanisms whereby the flow of molecules into and out of a cell is regulated. In the inner ear, a number of channels have been found to be critical to maintaining homeostasis. These include gap junctions, ion channels, and tight junctions. In addition, genes coding for neuronal synapses and integral membrane proteins have also been identified as key components of homeostasis.

**Gap Junctions**

Gap junctions are composed of transmembrane proteins called connexins. Six connexins combine to form a hemi-channel known as a connexon. When two connexons are joined, a gap junction is created. Within the inner ear, gap junctions allow the recycling of potassium ions back into the endolymph during mechanosensory transduction process. Mutations in connexins that alter the structure and/or function of the gap junctions thereby result in various forms of deafness. Currently, 3 genes have been identified which code for connexins, and which when mutated result in nonsyndromic hearing loss: GJB2, GJB3, and GJB6\(^{[91]}\) whereas GJB4 and GJA were found to associated with hearing loss, with unclear mechanisms.

**GJB2 (DFNB1A, DFNA3A)**

Gap junction protein beta 2 gene (GJB2) codes for connexin 26 and is located at chromosome 13 q11-12. GJB2 contribute to up to 50% of recessive nonsyndromic hearing loss\(^{[22]}\). While more than 100 mutations have been reported, the most common mutation in Europe, North America, and the Mediterranean is a deletion of 6 guanine nucleotides referred to as 35delG. Other prevalent mutations include the 167delT in the Ashkenazi Jewish population, 235delC in the Asian population and V37I in the Taiwanese population. The Indonesian population appears to lack GJB2 mutations in their deaf community\(^{[110]}\).

The recessive form, DFNB1, usually presents as a stable, severe to profound hearing loss; however, progression has been reported. While there is phenotypic variation across GJB2 genotypes, patients with the 35delG mutation tend to have the most severe form of hearing impairment\(^{[108]}\). Onset is almost always prelingual, but not necessarily congenital\(^{[21]}\). In fact, hearing may be normal at birth but progress rapidly during infancy\(^{[34]}\). Children have been diagnosed as late as 60 months due to nonpenetration at birth\(^{[79]}\). Thus, newborn screening tests may not always be reliable for detection and a role for genetic screening is recommended. The V37I is another GJB2 variant that is frequently found in East Asian populations and is considered as a genetic risk-indicator of postnatal permanent childhood hearing impairment and is associated
with mild to moderate hearing loss\textsuperscript{[138]}. The autosomal dominant form, DFNA3, typically presents during childhood as a progressive, moderate to severe hearing impairment affecting mostly high frequencies. It progresses to involve middle frequencies by middle age\textsuperscript{[21]}. As with recessive forms of GJB2, phenotypic variability is present in terms of the severity and evolution of hearing impairment\textsuperscript{[28]}. DFNA3 has been associated with variable degrees of hereditary palmoplantar keratoderma. The association presumably relates to the expression of connexin 26 in both support cells within the cochlea and in epidermis cells of the palms and soles\textsuperscript{[59]}. 

**GJB3 (DFNA2B)**

Gap junction protein beta 3 (GJB3) gene is located on chromosome 1p34. The gene codes for connexin 31 and mutations result in an autosomal dominant inheritance pattern of nonsyndromic hearing loss\textsuperscript{[57]}. While recent studies have examined functional impact of GJB3 mutations, their pathogenic role in the pathogenesis of deafness remain unclear\textsuperscript{[156]}. In the developing otocyst epithelium of mouse, Gjb3 is expressed in various subtypes of fibrocytes, either within the spiral limbus or along the spiral ligament, as well as in the basilar membrane cells, in the Reissner’s membrane cells, and in subsets of the cellular elements of the cochlear ganglion\textsuperscript{[58]}. Evidence exists that mutations of GJB2 and GJB3, coding for connexin 26 and connexin 31 respectively, can interact to cause hearing loss in digenic heterozygous individuals\textsuperscript{[140]}. Clinically, DFNA2 presents as a postlingual, high frequency hearing loss with onset between 20 and 40 years of age\textsuperscript{[5]}. An association with auditory and peripheral neuropathies has also been demonstrated\textsuperscript{[48]}. Moreover, GJB3 mutations have also been linked to erythrokeratoderma variabilis without any signs of hearing impairment\textsuperscript{[59]}. 

**GJB6 (DFNB1B, DFNA3B)**

The gap junction protein beta 6 gene codes for connexin 30 and is located on chromosome 13q12. Deficiencies in connexin 30 have been shown to disrupt the intrastrial fluid-blood barrier within the cochlear stria vascularis\textsuperscript{[54]}. GJB6 mutations are associated with both nonsyndromic deafness of dominant and recessive inheritance phenotypes. These phenotypes may have a monogenic or digenic pattern, caused by mutations in GJB6 or GJB2, or a combination of the two\textsuperscript{[18]}. The autosomal dominant form, DFNA3, affects middle to high frequencies and progresses to become more severe; it can be either pre- or postlingual \textsuperscript{[134]}. The autosomal recessive form, DFNB1, is characterized by a congenital, mild-to-profound deafness without progression\textsuperscript{[56]}. 

**Ion channels**

**KCNQ4 (DFNA2)**

The potassium voltage-gated channel, KQT-like subfamily, member 4 gene (KCNQ4) mutations have been reported as a cause of autosomal dominant hearing loss. The clinical picture is similar to that produced by GJB2 mutations; characterized by a postlingual, progressive, high-frequency hearing loss\textsuperscript{[59]}. The earliest age of onset reported in the literature is 5 years\textsuperscript{[99]}. The gene is located at chromosome 1p34 and encodes the voltage-gated potassium Kv7.4 channel protein\textsuperscript{[25]}. At young ages, hearing loss in DFNA2 patients tends to be moderate and predominantly affects high frequencies. The hearing loss progresses, usually in less than 10 years, to more than 60 dB with middle and low frequencies also involved. By the age of 70, all affected individuals in DFNA2 families have severe to profound hearing loss across all frequencies\textsuperscript{[52]}. Mutations in KCNQ4 cause deafness by presumably impairing cell-surface potassium channel expression\textsuperscript{[69]}. A recent study reports that two mechanisms, including decreased cell surface expression and impaired conductance of the KCNQ4 channel are underlying hearing loss in DFNA2\textsuperscript{[52]}.

**SLC26A4 (DFNB4)**

Solute carrier family 26, member 4 (SLC26A4) encodes a pendrin protein on chromosome 7q31. In the inner ear, Pendrin functions as a chloride-iodide transporter\textsuperscript{[106]}, thereby controlling the pH of endolymph and allowing for proper function of certain potassium and calcium channels. About 150 different SLC26A4 gene mutations have been found in patients with PDS and nonsyndromic deafness (http://www.healthcare.uiowa.edu/labs/pendredandbor//slcMutations.htm). In fact, it may be the second most common cause of nonsyndromic hereditary hearing loss in the Caucasian population\textsuperscript{[9]}. Without proper protein function, associated structural changes occur in the inner ear including enlargement of the vestibular aqueduct and of the endolymph sac\textsuperscript{[60]}. These changes are frequently seen in patients with DFNB4 and are helpful in identifying the etiology of hearing loss. The pattern of hearing loss in DFNB4 is prelingual and sloping with profound high frequency hearing loss\textsuperscript{[9]}. DFNB4 is allelic with Pendred Syndrome (congenital deafness and goiter); While individuals with Pendred syndrome have complete loss of chloride and iodide transport, DFNB4 individuals have enough residual function to preclude goiter formation\textsuperscript{[105]}.

**Tight Junctions**

**CLDN14 (DFNB29)**

The Claudin 14 gene (CLDN14) is located on 21q22.3\textsuperscript{[58]} and encodes a tight junction protein that maintains ionic composition of fluid at the basolateral surface of outer hair cells\textsuperscript{[6]}. CLDN14 mutations prohibit the resulting protein from forming tight junctions, a function that is critical for the hearing process. The absence of Claudin
14 from tight junctions in the organ of Corti leads to altered ionic permeability of the paracellular barrier of the reticular lamina and that prolonged exposure of the basolateral membranes of outer hair cells to high potassium concentrations may be the cause of the death of hair cells. Mutations at the DFNB29 locus generally produce a typical autosomal recessive picture of hearing loss—prelingual, bilateral, moderate to profound sensorineural hearing loss affecting all frequencies\[93\].

**Auditory Neuron Synapse**

**OTOF (DFNB9)**

The Otoferlin gene (OTOF) is located on chromosome 2p23.1 \[137\]. The gene codes for otoferlin which is a member of the mammalian ferlin family of membrane-anchored cytosolic proteins. It is thought to play a role in the composition of ribbon synaptic vesicles and thereby affect neurotransmitter release at the inner hair cell of cochlea. Mutations in OTOF cause DFNB9 recessive deafness\[98\]. DFNB9 presents as hearing loss that is prelingual and profound \[99\]; this recessive hearing loss is unique in that it may have an associated auditory neuropathy whereby otoacoustic emissions are normal while auditory brainstem responses remain absent or grossly abnormal\[97\]. OTOF gene mutations have been inferred to be responsible for 2-3% non-syndromic hearing losses (NSHL) in some ethnic groups, and most of them meet the diagnostic criteria for autosomal nonsyndromic deafness (ANSD)\[90\].

**PJVK (DFNB59)**

The PJVK gene encodes the protein PJVK which belongs to the gadermin family. The encoded protein is found only in vertebrates and required for the proper function of auditory pathway neurons. Defects in this gene are a cause of DFNB59. Homozygous PJVK-mutated knock-in mice showed mimic phenotype of patients with PJVK mutations, such as abnormal ABR and preserved function of IHCs (inner hair cells), and OHCs (outer hair cells)\[90\]. DFNB59 was the first reported gene that leads to deafness via neuronal dysfunction along the auditory cascade. Several mutations have been found in the PJVK gene\[130,36\]. The p.R183W mutation was found in three Iranian families with nonsyndromic deafness due to a neuronal defect and in a Turkish family without transiently evoked otoacoustic emissions\[20,18\]. Haplotype analysis did not suggest a founder effect for the Turkish and Iranian families with the mutation. At the same position, the p.R183Q was detected in one Chinese patient with ANSD \[119\] suggesting pathogenicity of the variants.

**Integral Membrane Proteins**

**TMC1 (DFNB7, DFNB11, DFNA36)**

The transmembrane Channel-Like 1 (TMC1) gene is located on 9q21.12 \[49\]. Allelic mutations may result in either autosomal dominant or autosomal recessive nonsyndromic hearing loss. The autosomal recessive loci DFNB7 and DFNB11 are characterized by a prelingual, profound hearing loss phenotype; the autosomal dominant allele, DFNA36 results in a postlingual progressive hearing loss that initially affects high frequencies and eventually extends to all frequencies; a later onset of hearing impairment is associated with slower progression in DFNA36 individuals \[86\]. TMC1 is a transmembrane protein that may have a role in postnatal hair cell development or maintenance \[99\], but its exact function is not known.

**TMIE (DFNB6)**

The transmembrane Inner Ear (TMIE) gene is also predicted to encode a transmembrane protein without a clearly known function. Mutations in TMIE result in DFNB6 deafness\[77\]. A study of the recessive Tmie mutation in mouse model showed shortened stereocilia of outer hair cells, which suggests that Tmie might influence actin filament dynamics in the normal hair bundle or alternatively plays a role in the organization of cytoskeleton-membrane interactions in sensory hair cells\[140\]. Mutation in TMIE causes an autosomal recessive prelingual deafness that profoundly affects all frequencies\[101\]. The circling mouse with TMIE gene deletion has deafness and exhibits abnormal behaviors including circling and hyperactivity. The hair cells in circling mice demonstrate abnormal maturation and mechanotransduction \[82\].

**Hair Cell Structure**

The structure of a hair cell is largely determinant of its ability to function properly. Thus, alterations in structure will result in various forms of hearing loss. The hair cell structure is maintained by adhesion proteins, ciliary proteins, as well as myosins.

**CDH23 (DFNB12)**

Mutations in cadherin 23 (CDH23) cause autosomal recessive, nonsyndromic deafness DFNB12. Affected individuals present with prelingual, moderate to profound sensorineural hearing loss (SNHL) without any vestibular impairment\[13\]. The DFNB12 locus maps to chromosome 10q21-q22\[12\]. It is allelic with Usher Syndrome type 1D (profound congenital deafness, variable retinitis pigmentosa, and variable vestibular dysfunction). The difference between the syndromic and nonsyndromic presentation depends on the type of allelic mutation, whereby all the nonsyndromic cases are caused by a missense substitution \[128\]. The CDH23 encodes the protein cadherin which interacts with harmonin b, a macromolecular organizer, to form a transmembrane complex that connects
stereocilia into a bundle. Furthermore, CDH23 interacts with protocadherin 15 (PCDH15) to form tip links that connect stereocilia and control mechanoelectric transduction in the inner ear.

Ciliary Structure

**STRC (DFNB16)**

The stereocilin (STRC) gene encodes the protein stereocilin which is associated with the hair bundles of stereocilia within the inner ear. It is located on chromosome 15q15 and defines the autosomal recessive DFNB16 deafness locus. Mutations in STRC result in two possible phenotypes. A first family was reported to have a non-progressive deafness that presented in early childhood with moderate impairment at low and middle frequencies and severe impairment at high frequencies whereas Verpy et al., (2001) reported a separate family with DFNB16 deafness that had profound prelingual SNHL. Finally, the STRC gene is thought to be a major contributor to nonsyndromic bilateral sensorineural hearing loss (NBSNHI) among the GJB2 mutation negative probands, especially in those with mild to moderate hearing impairment.

**Myosins**

Myosins are a family of motor proteins that hydrolyze ATP to produce actin-based motility. There are over 30 families of myosins, each differentiated by its ATP-hydrolyzing domain. Within the inner ear, myosins are involved in the structure and movement of stereocilia. Myosin mutations that alter the function of stereocilia result in sensorineural hearing loss. Mutations in MYO3A, MYO6, MYO7A, MYO1C, and MYH9 have been reported to cause nonsyndromic hearing loss.

**MYO3A (DFNB30)**

Myosin IIIA (MYO3A) is thought to function as a motor at the tip of stereocilia. The gene has been mapped to chromosome 10p11.1, and is responsible for the DFNB30 locus. Mutations in MYO3A produce a progressive hearing loss, which first affects the high frequencies. The impairment begins in the second decade and progresses over about 30 years to become severe at high and middle frequencies and moderate at low frequencies. The phenotype is thought to be inherited in an autosomal recessive fashion, although autosomal dominant inheritance with variable penetrance has not been ruled out. In the Myo3a knockin KI/KI mice model, the unique N-terminal kinase domain and a C-terminal actin-binding domain of MYO IIIA, which localizes to the tips of stereocilia, is absent. Hearing loss, as measured by auditory brainstem response, is reduced and progresses significantly with age. Outer hair cells of Myo3a KI/KI mice degenerate with age in a pattern consistent with their progressive hearing loss.

**MYO6 (DFNB37, DFNA22)**

Myosin VI (MYO6) is a class of myosins that move toward the minus end of actin filaments, in the opposite direction that other characterized myosins move. Its functions in the transportation of intracellular vesicles and organelles, to facilitate the removal of molecular components that are released by treadmilling at the taper of the stereocilium. The MYO6 gene has been mapped to chromosome 6q13. Mutations in MYO6 have been documented to produce both autosomal dominant and autosomal recessive forms of nonsyndromic deafness that affect all frequencies. The recessive form, DFNB37 deafness, is congenital and profound, whereas the dominant DFNA22 deafness is postlingual, late onset, and progressive and has a phenotype that mimics presbycusis. Additionally, mutation in MYO6 has also been associated with familial hypertrophic cardiomyopathy. This feature may prove useful in distinguishing DFNA22 from other nonsyndromic autosomal dominant hearing loss genes.

**MYO7A (DFNA11, DFNB2)**

Myosin VII A (MYO7A) is located on chromosome 11q12-21, designated DFNA11. A second locus (DFNB2) has been more within the MYO7A interval, at 11q13.5. Deafness in DFNA11 is postlingual and progressive with onset in the first to second decade of life that affects all frequencies. DFNB2 deafness can be prelingual, progressive, and severe-to-profound or it may have variable age of onset as a severe hearing loss. In most cases, MYO7A is associated with vestibular dysfunction, ranging from vertigo to complete absence of vestibular function. The MYO7A mutation analysis is believed to be valid for the early diagnosis of Usher syndrome before the appearance of visual symptoms. MYO7A is part of a functional unit, along with harmonin b and cadherin 23, that is essential to ensure stereocilia cohesion. The syndromic and nonsyndromic forms may lie on the same phenotype spectrum, whereby the presence of some residual protein function results in a less severe phenotype in nonsyndromic deafness.

**MYO15A (DFNB3)**

Myosin XVA (MYO15A) responsible for the DFNB3 locus is located on chromosome 17p11.2. MYO15A transports the protein whirlin and perhaps other molecular components to their normal location at the stereocilia tip for programmed elongation of the stereocilia in hair cells of cochlea. Without its proper function, affected individuals have an autosomal recessive prelingual deafness, that may be ranked as moderate, severe or profound. The shaker2 (sh2) mouse is a murine model for human non-syndromic deafness DFNB3. In this mouse model,
the BAC transgene correction confers hearing capability, normal cochlear structure and function in 6-month-old Myo15a mutant mice. In addition, excess Myo15a expression has no physiologically significant protective or deleterious effects on hearing of normal mice, suggesting that the dosage of Myo15a may not be problematic for gene therapy\[43\].

**Transcription Factors**

Transcription factors are proteins that bind to DNA domains and help initiate a program of increased or decreased gene transcription. They play important roles in a cell’s maintenance, development, response to neighboring cells, and response to the environment of the cell.

The following transcription factors and/or activators have been implicated in nonsyndromic sensorineural hearing loss.

**EYA4 (DFNA10)**

Eyes absent homolog 4 (EYA4) was named for its counterpart in Drosophilia. The human EYA4 maps to chromosome 6q23\[7\] and is responsible for hearing loss at the DFNA10 locus\[120\]. EYA4 is a transcriptional activator that increases the rate of gene transcription in cells of the inner ear. Mutations in EYA4 result in an autosomal dominant pattern of progressive deafness that begins between the ages of 20-60 and affects all frequencies. The onset of DFNA10 deafness is later than most autosomal dominant deafness phenotypes. Additionally, DFNA10 deafness may be associated with a dilated cardiomyopathy which is unique to this phenotype\[103\]; the presence of cardiomyopathy seems to correlate with EYA4 mutation position\[62\].

**POU4F3 (DFNA15)**

POU4F3 is a member of the family of POU domain transcription factors. This group of proteins shares a POU-specific domain and a POU homeodomain, both of which are required for high-affinity binding to DNA target sites. POU4F3 is located on chromosome 5q31 and defines the DFNA15 locus. The target gene for POU4F3 is the Growth Factor Independent 1 (GFI1) gene\[19\]. Affected individuals demonstrate autosomal dominant inheritance of progressive hearing loss. Onset is between the ages of 15 and 30 and impairment becomes moderate to severe across all frequencies by age 50\[154\]. It should be noted, however, that some cases have shown a highly variable phenotypic profile with respect to onset, progression, and affected frequencies\[15,31,149\]. Full penetrance has been documented for this gene mutation.

**POU3F4 (DFNX2)**

POU domain 3 factor 4 (POU3F4) is a transcription factor gene responsible for an X-linked pattern of deafness, known as DFNX2 deafness\[89\]. The POU3F4 gene is located on chromosome Xq21.1\[24\]. Affected individuals have a characteristic computerized tomography (CT) appearance of a dilated internal auditory canal along with temporal bone abnormalities that can range in severity. Regardless, the sensorineural component progresses over time and the deafness may present alongside vestibular dysfunction\[115\].

**Cytokinesis**

**DIAPH1 (DFNA1)**

Diaphenous homolog 1 (DIAPH1) gene mutations is known to cause an autosomal dominant, progressive, postlingual DFNA1 sensorineural hearing loss (SNHL) that begins with increased thresholds at low frequencies and progresses to involve all frequencies\[81\]. The hearing loss begins around the age of 10 and progresses to profound deafness by age of 30. The gene locus has been mapped to 5q31\[139\]. DIAPH1 belongs to the formin family of proteins. It is believed to be involved in cytokinesis and establishment of cell polarity and is a profilin ligand and target of Rho that regulates polymerization of actin, the major component of the cytoskeleton of hair cells of the cochlea\[60\], and it is thus thought to be essential to the structure and function of the stereocilia. Although it is expressed in many tissues, DFNA1 individuals do not have any other symptoms or any vestibular dysfunction.

**ESPN**

The ESPN (ESPN) codes for the Espin protein, which is believed to function in the growth and maintenance of stereocilia and has a potent actin bundling activity in the inner ear\[23\]. It maps to chromosome 1p36.31-36.11 and defines the autosomal recessive DFNB36 deafness locus. Affected individuals have a prelingual, profound hearing impairment associated with vestibular areflexia\[78\]. Boulouiz et al. (2008) showed that mutations in the ESPN gene could cause recessive nonsyndromic deafness without vestibular areflexia.

The vestibular dysfunction may or may not be symptomatic. However, if areflexia is confirmed then it becomes helpful in narrowing the differential of which deafness genes may be involved in the pathology. ESPN mutations have also been shown in individuals with autosomal dominant nonsyndromic hearing loss without any vestibular involvement\[25\]. Boulouiz et al. (2008) mapped the disease locus in a large consanguineous ARNSHL
family from Morocco by genome-wide linkage analysis to the DFNB36 locus and a recessive ESPN mutation causing congenital hearing loss, but without vestibular dysfunction.

**Extracellular Matrix Proteins**

The extracellular matrix (ECM) of the auditory system optimizes the propagation and detection of sound within the Cochlea. It is especially important for the tectorial and basilar membrane which physically move as auditory stimuli are transmitted. Changes in the ECM may affect these membranes resulting in sensorineural hearing loss.

**COL11A2 (DFNA13, DFNB53)**

Mutation in the collagen type 11 alpha 2 gene (COL11A2) results in autosomal dominant hearing loss at the DFNA13 locus located on chromosome 6p21.3. It is allelic with Stickler syndrome, i.e. short stature, myopia, arthropathy, mid-face hypoplasia along with high frequency progressive sensorineural hearing loss. In contrast to Stickler syndrome, DFNA13 is a postlingual mid-frequency hearing loss that begins at age 20-40 years and does not progress beyond presbycusis. Thus the audiogram of affected individuals is initially U-shaped (‘cookie-bite’) and eventually flattens with presbycusis. Mutation in COL11A2 can also cause autosomal recessive non-syndromic hearing loss at the DFNB53 locus, characterized by a prelingual, profound, non-progressive hearing loss phenotype. COL11A2 codes for collagen fibrils that form the structure of the tectorial membrane. Many collagen diseases are associated with hearing loss, so it may be that nonsyndromic hearing loss is simply the mildest phenotype associated with mutations in COL11A2.

**COCH (DFNA9)**

The coagulant factor C homolog (COCH) gene, mapped to chromosome 14q12-13, is associated with the DFNA9 locus. It is inherited in an autosomal dominant fashion and presents as postlingual, progressive hearing loss, beginning at high frequencies and progressing over 10-20 years to profound hearing loss at all frequencies. Age of onset varies between 20 to 60 years. The earliest reported age of onset is two years. The clinical sequence has started in the child with a vestibular dysfunction: recurrent rotatory dizziness during 12 months. And At the age of 3, he developed sensorineural hearing loss on both sides. Individuals with DFNA9 deafness also have vestibular dysfunction similar to Meniere’s disease. Patients often self report the vestibular disturbances, which becomes helpful in identifying this type of non-syndromic hearing loss. A new study describes newly discovered extralabyrinthine findings within the middle ear in DFNA9 and discusses their implications.

COCH encodes the protein cochlin, which is abundantly expressed both in the cochlea and in the vestibular labyrinth. Cochlin is likely synthesized in the endoplasmic reticulum and secreted into the extracellular space where it plays an integral part in mediating interactions among proteins. Mutations in COCH may disrupt these protein interactions thereby altering the function of the extracellular matrix. DFNA9 may not be caused by haploinsufficiency of COCH, rather a gain-of-function or dominant-negative mutation.

**TECTA (DFNA12, DFNB21)**

Mutations in the alpha tectorin (TECTA) gene cause hearing loss that is unique because it is a form of autosomal-dominant, congenital hearing loss. Typically autosomal-dominant hearing impairment is not present at birth but TECTA is the exception. TECTA mutations produce moderate to severe, nonprogressive hearing loss affecting predominantly middle frequencies. The phenotype is linked to loci DFNA12 at chromosome 11q22-24. An autosomal recessive form of deafness has also been linked to TECTA at the DFNB21 locus. It presents as prelingual, severe hearing loss with a flat or U-shaped audiogram. This audiometric profile is easily recognizable among families with autosomal recessive, non-syndromic hearing impairment due to the dip in the middle frequencies. A study for the prevalence of hearing loss caused by TECTA mutations has been investigated in Japanese families with autosomal dominant hearing loss. TECTA mutations were detected in 2.9% (4/139) of those families.

The TECTA gene encodes a major non-collagenous component of the tectorial membrane called alpha tectorin. Alpha tectorin bridges the stereocilia bundles of the hair cells, allowing them to move in unison. Ultimately, mutations in TECTA disrupt sound transmission to the stereocilia.

**Mitochondrial Deafness**

Mitochondria are intracellular organelles that not only produce cellular energy in the form of ATP, but also contribute to cell-mediated death, or apoptosis, and are implicated in preventing cell damage by reactive oxygen species. Mitochondria harbor their own DNA that is inherited maternally and encodes proteins necessary for their structure and function. Mutations in mitochondrial DNA (mtDNA) cause a variety of systemic disorders including syndromic hearing loss; additionally, a number
of mtDNA mutations have been identified which result in nonsyndromic sensorineural hearing loss. In particular, mutations in two genes, MTRNR1 and MTTS1, have been commonly found to result in nonsyndromic deafness.

**MTRNR1**

MTRNR1 encodes for the 12s ribosomal RNA (rRNA). Mutations in this gene result in a maternally inherited nonsyndromic nonsyndromic hearing loss that is often provoked or made worse by exposure to aminoglycoside antibiotics [89]. Certain mutations in MTRNR1 may cause tighter binding to aminoglycosides causing hypersensitivity to the antibiotic that become toxic to hair cells [40]. There is also evidence for the fact that in individuals with an MTRNR1 mutation, aminoglycoside reduce mitochondrial protein synthesis precluding normal cellular function [137].

The most common mutation in MTRNR1 is the A1555G mutation. This mutation has been found in many families with maternally-inherited hearing loss as well as in individuals with aminoglycoside-induced hearing loss. Penetration can be variable [10], low penetrance has been specifically reported among the Chinese population [17]. Because of the variable penetrance, a range of phenotypes from normal hearing to profound deafness exist and imply the presence of a nuclear modifier [54,142]. In 2006, tRNA 5-methylaminomethyl-2-thiouridylate methyltransferase (TRMU) was identified as the modifier gene [55]. When mutated, TRMU alters the phenotypic expression of the A1555G mutation in MTRNR1. This may be due to variable exposure to aminoglycosides between family members. In fact, age of onset also varies, in which case the onset of deafness in exposed individuals is younger [138]. Moreover, associated symptoms have occasionally been linked to A1555G-related hearing loss, including Parkinson Disease and cardiomyopathy [100]. Nonetheless, the majority of cases are nonsyndromic.

**MTTS1**

The MTTS1 gene encodes the mitochondrial tRNA for serine (UCN) (tRNAser(UCN)). Several nonsyndromic progressive hearing loss causing mutations have been identified in MTTS1. Similarly to mutations in MTRNR1, penetrance due to MTSS1 mutation varies greatly both within and between families. Additionally, certain mutations can cause associated features; the A7445G mutation has been associated with palmar-plantar keratoderma [89] and the 7472insC mutation has been linked to dysarthria, ataxia, myoclonus, and cognitive impairment [41]. Mutations in MTTS1 have also been shown to induce aminoglycoside hypersensitivity as seen with MTRNR1 mutations [42]. In essence, due the relationship to aminoglycoside antibiotics, a role of the screening of mitochondrial mutations in families with maternally-inherited sensorineural hearing loss is warranted.

**Other/Unknown Function**

**TMPRSS3 (DFNB8, DFNB10)**

Mutations in transmembrane protease, serine 3 (TMPRSS3) are associated with two loci that can be differentiated by phenotype. DFNB8 begins in childhood (age 10 to 12) and rapidly progresses over 5 year to profound deafness across all frequencies [151], DFNB10 on the other hand is typically present at birth as severe SNHL but individuals can present as late as 6 or 7 years of age [80]. The TMPRSS3 gene is located on chromosome 21q22 [151]. TMPRSS3 is mainly expressed in the spiral ganglion neurons and is involved in the process of aminoglycoside antibiotics induced deafness [180]. It has also been suggested that lack of Tmprss3 leads to a decrease in Kcnma1 potassium channels expression in inner hair cells [73].

**WFS1 (DFNA6/14/38)**

Defects in the Wolfram syndrome 1 gene (WFS1) is one of only two genes that lead to a nonsyndromic, autosomal dominant low frequency sensorineural hearing loss. Mutations in WFS1 have been linked to DFNA6, DFNA14 and the DFNA38 locus. Other than DFNA1, the WFS1 loci DFNA6/14 can be singled out by its S-shaped pure tone audiogram [121]. DFNA6/14/38 deafness has minimal progression, consistent with changes of presbycusis. This can be contrasted with DFNA1 which has a rapid progression [80]. Furthermore, the onset of DFNA14/6/38 is in childhood, from 5 to 15 years of age [122]. WFS1 is located on chromosome 4p16 [111]. It codes for an extracellular matrix-integral glycoprotein of endoplasmic reticulum, suggesting thus a role in membrane trafficking, protein processing and regulation of endoplasmic reticulum calcium homeostasis [113]. WFS1 mutations are also responsible for a syndromic form of hearing loss, Wolfram syndrome, defined by diabetes insipidus, diabetes mellitus, optic atrophy, and sensorineural deafness. The unique collection of audiometric data from genotyped Wolfram syndrome patients shows no substantial progression in sensorineural hearing impairment with advancing age, no relation to the types of WFS1 mutations identified, and no significant sex-related differences [87].

**PCDH15 (DFNB23)**

The protocadherin 15 (PCDH15) gene on chromosome 10q21.1 is responsible for DFNB23. PCDH15 interacts with cadherin 23 to form tip links of stereocilia [60].
Missense mutations in PCDH15 have been found to cause DFNB23. It has a classic autosomal recessive hearing loss phenotype that is a prelingual severe to profound sensorineural deafness. The gene is allelic with Usher Syndrome 1F, caused by more severe mutations (splicing, frameshift, nonsense, large deletions) producing a syndromic phenotype which also involves visual impairment [1].

**USH1C (DFNB18)**

Mutations in the Usher Syndrome 1C (USH1C) gene can also lead either to a syndromic or a nonsyndromic phenotype depending on the severity of the mutation. The nonsyndromic phenotype, DFNB18, is a prelingual profound deafness. Usher 1C syndrome not only has profound prelingual deafness, but also retinitis pigmentosa and vestibular areflexia [81]. Both phenotypes are inherited in an autosomal recessive fashion. The USH1C gene is located on chromosome11p14.3, and codes for the protein harmonin, a PDZ domain protein that forms a complex with CDH23 to bundle stereocilia [107].

**WHRN (DFNB31)**

Mutations in the whirlin (WHRN) gene, mapped on 9q32-q34 is responsible for prelingual profound deafness (DFNB31) [15]. Whirlin encoded by the WHRN gene is known to interact with MYO15A for stereocilia morphogenesis [130]. Mouse studies show that WHRN is involved directly in actin filament packing that is necessary for the elongation of stereocilia after birth [91]. While Myosin XV may interact with other proteins, Whirlin is important for hair cell maturation [130]. WHRN mutations may also cause Usher Syndrome type 2D (USH2D) which presents with deafness, retinitis pigmentosa, but normal vestibular function. Mutations to the long isoform are believed to cause the syndromic manifestations [115].

**Genomic Advances for Molecular Testing and Gene Discovery in Hereditary Hearing Loss**

Testing for mutations responsible for the most common forms of syndromic and non-syndromic hearing loss plus congenital CMV infection can determine the cause of hearing loss in most cases. Approximately 15%-25% of congenital hearing loss cases in children are due to Cytomegalovirus (CMV) infections. Early identification of deafness allows for appropriate intervention or rehabilitation that has lifelong implications for language development. The lifetime societal costs for childhood hearing loss are estimated at $1.1 million (http://www.audiologyonline.com). With over 90% of babies currently being screened for hearing loss at birth in the U. S. and given the recommendations to incorporate genetic testing as part of expanded Early Hearing Detection and Intervention (EHDI) programs, the Joint Committee on Infant Hearing (JCIH) endorses early detection of and intervention for infants with hearing loss through integrated, interdisciplinary community, state, and federal systems of universal newborn hearing screening. Furthermore, since several hundred genes are involved in the biology of hearing loss and additional genes and mutations may yet be identified, newer platform technologies for high-throughput genetic testing should make diagnostic testing for hearing loss more flexible, less expensive, and more comprehensive while being as sensitive.

**Arrayed Primer Extension Microarray for Detection of Known Deafness-Associated Mutations**

Microarray approaches have been used extensively for analyzing the expression levels of thousands of genes and for performing multiplex genotyping of single nucleotide polymorphisms (SNPs) and large-scale mutation screening. The DNA microarray, or biochip, is a hybridization-based genotyping technique that offers simultaneous analysis of many genetic mutations. Several studies using microarray diagnostics for high-throughput, comprehensive, and cost-effective molecular screening of mutations of deafness genes have demonstrated accurate and reliable results [109,33]. The HHL APEX (Hereditary Hearing Loss Arrayed Primer Extension) microarray based on APEX designs [90] was the first hearing loss diagnostic panel using this strategy. APEX array technology allows the simultaneous analysis of multiple mutations through hybridization of fragmented template DNA to specifically designed primers, followed by single nucleotide extension at the site of each mutation. The HHL APEX array enables analysis of 198 mutations across eight genes (GJB2, GJB6, GJB3, GJA1, SLC26A4, SLC26A5, MTRNR1 and MTTS1) most commonly associated with non-syndromic sensorineural hearing loss, in a single test. All known mutations for each of the 8 genes are included on the APEX microarray at the time of its design. However, novel mutations continue to be identified and thus lack of identification of mutations on the HHL array, therefore, does not necessarily exclude a gene as the cause of the hearing loss. Hence, targeted diagnostic sequencing still remains a follow-up tool to screening with the APEX assay, for patients in whom a single pathogenic mutation was identified in a gene on the array. Furthermore, since the APEX panel is comprised of a limited number of genes and sequence variants, it may also not accurately represent the most frequent hearing loss al-
leles in a given test population. Together, the current APEX array configuration adds little diagnostic value for patients with no mutations in the GJB2 and/or GJB6 genes \(^{(49)}\). The mutations on the APEX array do not substantially contribute to age-related hearing loss either \(^{(50)}\).

Li et al (2008) combined allele-specific PCR and universal array methodologies for the detection of mutations causing hereditary hearing loss. The approach termed multiplex allele-specific PCR-based universal array (ASPUA) allows for simultaneous screening of 11 mutations in 3 genes. These include GJB2 (c.35delG, c.167delT, c.176_191del16, c.235delC, c.299_300delAT), GJB3 (c.538C>T; p.Arg180X, c.547G>A; p.Glu183Lys) and SLC26A4 (c.707T>C; p.Leu236Pro, c.2168A>G; p.His723Arg, c.919-2A>G; Splice acceptor). The ASPUA universal array makes the multiplex detection flexible, as the numbers of mutations detected can be increased and the microarray could also be redesigned with a greater number of subarrays so that much larger numbers of patient samples could be tested.

**High-Throughput Detection of Deafness Causing Mutations Using Resequencing Microarrays**

Two high-throughput resequencing Affymetrix arrays capable of resequencing 13 genes involved in sensorineural hearing loss (SNHL) (GJB2, GJB6, CDH23, KCNE1, KCNQ1, MYO7A, OTOF, PDS, MYO6, SLC26A5, TMIE, TMPRSS3, USH1C) were developed by research groups from Harvard and from Cincinnati \(^{(47)}\). This oligo-hybridization array-based sequencing methodology was used in the design of OtoChipTM Test for hearing loss and Usher Syndrome now available at Harvard Medical School. The current format of OtoChipTM contains a DNA sequence of 2750 base pairs encompassing 19 genes [http://pcpgm.partners.org/lmm/tests/hearing-loss/Otochip].

The HHL APEX array can only detect specific known hearing loss mutations whereas the Affymetrix resequencing arrays allow for the discovery of new mutations. However, resequencing arrays do not have high sensitivity for detection of small deletions and insertions, which constitute ~24% of disease-causing mutations in the Human Gene Mutation Database \(^{(47)}\).

**Comprehensive Genetic Testing for Hearing Loss Using Targeted Enrichment and Massively Parallel Sequencing.**

OtoSCOPE, a comprehensive genetic screen for deafness, was developed at the University of Iowa \(^{(47)}\). This diagnostic platform uses targeted capture and massively parallel sequencing to provide direct sequencing of the 66 genes known to cause NSHL. Usher syndrome and Pendred syndrome genes are also included [http://www.morl-otoscope.org]. OtoSCOPE offers relatively high specificity and sensitivity. However, its greatest limitation is that repetitive regions cannot be captured. Another clinical assay, testing 84 human genes involved in both syndromic HL and NSHL, is also available from Otogenetics [http://www.otogenetics.com/].

**Next Generation Sequencing to Identify Genes for Hereditary Hearing Loss.**

Now, the relatively low cost and rapid technological advances in next generation sequencing (NGS) technologies are dramatically changing our ability to identify genes and disease-causing mutations. Because of the genetic heterogeneity of hearing loss, targeted DNA capture and massively parallel sequencing are ideal tools to address this challenge. These new sequencing technologies replace the one small-template-at-a-time paradigm of Sanger sequencing with ‘massively parallel’ sequencing of millions of small fragments covering the whole genome as well as individual regions with great redundancy. The basic methodological steps of NGS include: a) template preparation, using methods ranging from pyrosequencing and sequencing by ligation to reversible dye termination and real-time single molecule sequencing; b) massive sequencing and imaging, and c) data analysis \(^{(64,70)}\). The NGS market is rapidly evolving with a large number of developments taking place to increase accuracy and speed, and reduce costs of sequencing. Several NGS platforms are now available, including the 454 GS FLX (Roche), SOLiD (Life Technologies) and the HiSeq series (Illumina). More recently, “bench-top” DNA sequencing instrumentation such as 454 GS Jr (Roche), MiSeq (Illumina) and IonTorrent Personal Genome Machine (PGM) from Life technologies have also been released, capable of sequencing in a matter of days.

Several studies have used a combination of targeted capture and NGS technology to identify deafness genes. Targeted genomic capture is performed on the linked region identified previously by genetic analysis followed by sequencing of the chromosomal region. On a larger scale, whole exome sequencing is extremely promising, as it screens the exons of all genes by capturing and sequencing the 1% of the human genome that codes for protein. Several Human Exome Sequence Capture kits are now available allowing library preparation with subsequent exome enrichment. These include the Agilent SureSelect Human All Exon Kit, the Illumina TruSeq Exome Enrichment Kit, the TargetSeq in-solution target enrichment kit from Life Tech/Applied Biosystems and SeqCap EZ Exome from Roche NimbleGen. Both specific genomic regions capture and whole exomes sequencing have been successful at identifying genes for both
non-syndromic and syndromic hearing loss (Table 3). Obstacles and challenges still remain, but the field is changing at a dramatic pace, making deafness gene discovery faster and more efficient and screening for genetic hearing loss more comprehensive.

**Conclusion**

A thorough evaluation of patients with nonsyndromic deafness is warranted in order to provide accurate treatment goals and may be beneficial for the future application of gene therapy in hearing loss. As can be seen, mutations in most nonsyndromic deafness genes present a classical clinical feature dependent on inheritance pattern. Autosomal recessive inheritance often implies a prelingual, severe deafness, whereas autosomal dominant inheritance implies postlingual onset and variable severity. There are some exceptions, however, for instance, mutations in MYO7A, TMPRSS3, and STRC are inherited recessively, but onset is postlingual. On the other hand, some dominantly inherited loci, like DFNA8/12, have prelingual onset. Mutations associated with loci DFNA12, DFNA13, and DFNA21, affect the middle frequencies thereby producing a U-shaped audiogram. Some defects, as exemplified by mutations in the POU3F4 gene, cause characteristic changes on imaging. A thorough evaluation of patients with nonsyndromic deafness is warranted in order to provide accurate treatment goals and may be beneficial for the future application of gene therapy in hearing loss. As can be seen, mutations in most nonsyndromic deafness genes present a classical clinical feature dependent on inheritance pattern. Autosomal recessive inheritance often implies a prelingual, severe deafness, whereas autosomal dominant inheritance implies postlingual onset and variable severity. There are some exceptions, however, for instance, mutations in MYO7A, TMPRSS3, and STRC are inherited recessively, but onset is postlingual. On the other hand, some dominantly inherited loci, like DFNA8/12, have prelingual onset. Mutations associated with loci DFNA12, DFNA13, and DFNA21, affect the middle frequencies thereby producing a U-shaped audiogram. Some defects, as exemplified by mutations in the POU3F4 gene, cause characteristic changes on imaging.

### Table 3

**Deafness Genes Discovered Using Targeted Capture and Next Generation Sequencing**

<table>
<thead>
<tr>
<th>Inheritance</th>
<th>Locus</th>
<th>Regions</th>
<th>Discoveries</th>
<th>Gene</th>
<th>Reference</th>
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<tbody>
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<td>TPRN</td>
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<td>Pierce et al., 2011</td>
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<td>Syndromic</td>
<td>3MC</td>
<td>Whole exome, 1.81 Mb analyzed, 3q27</td>
<td>MASPI</td>
<td></td>
<td>Sirmaci et al., 2010</td>
</tr>
<tr>
<td>Syndromic</td>
<td>HSAN1</td>
<td>Whole exome, 3.4 Mb analyzed, 19q13.2</td>
<td>DNMT1</td>
<td></td>
<td>Klein et al., 2011</td>
</tr>
</tbody>
</table>

**References**


Table 2  Nonsyndromic Deafness Loci with Unique Features

<table>
<thead>
<tr>
<th>Locus</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>DFNA1</td>
<td>Low frequency</td>
</tr>
<tr>
<td>DFNA2</td>
<td>Auditory and peripheral neuropathy; Erythrokeratodermia variabilis (No deafness)</td>
</tr>
<tr>
<td>DFNA3</td>
<td>Prelingual</td>
</tr>
<tr>
<td>DFNA6/14/38</td>
<td>Prelingual; Low frequency with minimal progression</td>
</tr>
<tr>
<td>DFNA9</td>
<td>Meniere–like symptoms</td>
</tr>
<tr>
<td>DFNA11</td>
<td>Asymptomatic vestibular dysfunction</td>
</tr>
<tr>
<td>DFNA12</td>
<td>Prelingual; Middle frequency</td>
</tr>
<tr>
<td>DFNA13</td>
<td>Middle frequency</td>
</tr>
<tr>
<td>DFNA17</td>
<td>Cochleosaccular degeneration</td>
</tr>
<tr>
<td>DFNA21</td>
<td>Middle frequency</td>
</tr>
<tr>
<td>DFNB2</td>
<td>Postlingual onset Reduced or absent vestibular function, vertigo</td>
</tr>
<tr>
<td>DFNB4</td>
<td>Dilated vestibular aqueduct or endolymphatic ducts</td>
</tr>
<tr>
<td>DFNB8/10</td>
<td>Postlingual onset</td>
</tr>
<tr>
<td>DFNB9</td>
<td>Auditory neuropathy</td>
</tr>
<tr>
<td>DFNB12</td>
<td>Atypical late onset retinitis pigmentosa; Borderline vestibular dysfunction</td>
</tr>
<tr>
<td>DFNB16</td>
<td>Postlingual onset</td>
</tr>
<tr>
<td>X–linked</td>
<td>Characteristic CT appearance</td>
</tr>
</tbody>
</table>


[102] Schneider ME, Dosé A, Salles FT, Chang W, Erickson FL,


[127] Zheng J, Miller KK, Yang T, Hildebrand MS, Shearer AE, DeLuca AP, Scheetz TE, Drummond J, Scherer SE, Legan PK, Goodyear RJ, Richardson GP, Cheatham MA, Smith RJ, Dallos P. Carcinoembryonic antigen-related cell adhesion molecule 16 interacts with alpha-tectorin and is mutated in autosomal dominant


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