Chapter 4

Advances in Genetic Diagnosis and Treatment of Hearing Loss — A Thirst for Revolution

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Additional information is available at the end of the chapter

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Abstract

Despite the significant advances in understanding the molecular basis of hearing loss, precise identification of genetic cause still presents some difficulties, owing to phenotypical variation. Gene discovery efforts for hearing disorders are complicated by extreme heterogeneity. Mutations in some of these genes, such as *GJB2, MYO7A, CDH23, OTOF, SLC26A4, TMC1*, are quite common and responsible for hearing loss. Clinical exome sequencing is a highly complex molecular test that analyzes the exons or coding regions of thousands of genes simultaneously, using next-generation sequencing techniques. The development of a biological method for the repair, regeneration, and replacement of hair cells of the damaged cochlea has the potential to restore normal hearing. At present, gene therapy and stem cells are two promising therapeutic applications for hearing disorders. Gene therapy and stem cell treatment have still a long way to go before these treatments will be available to use in humans. Therefore, existing measures must focus on the prevention of hearing loss to decrease the frequency of genetic hearing loss. Over time, genetic diagnostic tests will become available most rapidly, followed by targeted gene therapy or various permutations of progenitor cell transplantation, and eventually, the preventive interventions for a wider range of hearing impaired patients.

Keywords: Genetic hearing loss, next generation sequencing, genetic evaluation, gene therapy, stem cell therapy

1. Introduction

Genetic hearing loss has diverse etiologies and approximately 1% of all human genes are involved in the hearing process [1]. It is estimated that at least two-thirds of cases of childhood-onset hearing loss have a genetic cause [2], with the remaining one-third caused by environmental factors, e.g., cytomegalovirus infection, meningitis, acquired conductive loss, and the

impact of extracorporeal membrane oxygenation [3]. Many cases of later onset progressive hearing loss are genetic in origin, and genes also play an important role in progressive hearing loss associated with ageing. This chapter will deal with the ability to identify genetic problems or suspected genetic causes in hearing disorders, different types of gene mutation that causes deafness, genetic evaluation of hearing loss, special aspects of genetic tests such as next-generation sequencing, limitation of genetic testing, the development and evaluation of genetic treatment, management, prevention and genetic counseling, benefits of genetic research in deafness, and research needs/anticipating changes. It will conclude by the direction of possible future technological development in these aspects.

The majority of hearing loss is caused by mutations in the DNA (deoxyribonucleic acid) sequence of genes. There are four "bases" in a strand of DNA: adenine (A), guanine (G), thymine (T), and cytosine (C). The DNA also contains a monosaccharide sugar called deoxyribose and a phosphate group. According to base pairing rules (A with T, and C with G), hydrogen bonds bind bases of the two separate strands to make double-stranded DNA. Humans have approximately 30,000 genes. The DNA sequence of these genes provides the code for producing proteins (which consist of amino acids). The gene is located within a designated region on the chromosome and is composed of the different base pairs. The specific location on the chromosome where the gene is found is known as locus. For example, autosomal recessive deafness 1A (DFNB1A) is caused by mutation in the *GJB2* gene on chromosomal locus 13q11-q12. This means it is on the long arm of chromosome (q indicates the long arm) 13 somewhere in the range from band 1 of region 1 to band 2 of region 1 (11 or 12 represent the position on arm: 12-region 1, band 2). At times, changes (INDELs) occur in the DNA sequence of genes, such as a short segment of a gene is AGACATCATCTA and A has been replaced by a G at position 8 (AGACATCGTCTA) and C at position 4 has been deleted (AGAGATCGTTA), resulting in a mutation in the DNA sequence and affecting their functions such as a non-functioning protein may be produced or that protein may be missing altogether. If these mutations occur in a gene with important information for normal hearing, the result may be hearing loss, or in extreme cases, deafness.

2. Genetic causes of hearing loss

There are two types of hearing loss caused by genetics. About 30% of people with a genetic type of hearing loss are classified as syndromic, which involves other presenting abnormalities along with hearing impairment. Non-syndromic hearing loss occurs when there are no other problems associated with an individual other than hearing loss. From a genetic standpoint, this accounts for the other 70% of people, which attributes to the vast majority of genetic hearing loss.

The genetic basis is highly complex. There are many different ways that the DNA sequence of a gene can be changed, resulting in different types of mutation. The types of gene mutations include missence, nonsense, substitution, insertion, deletion, duplication, frameshifts, repeat expansion, splice site, and translocation. The chances of developing deafness caused by a

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mutated gene depend on whether the mutation is dominant or recessive. Dominant and recessive hearing loss results from the allelic mutation in some genes, syndromic and non-syndromic hearing loss is caused by mutations in the same gene, and recessive hearing loss may be caused by a combination of two mutations in different genes from the same functional group [4].

2.1. Non-syndromic hearing loss

The different gene loci for non-syndromic deafness are designated DFN (for DeaFNess). Based on the mode of inheritance, loci are named as A, B, X, and Y for autosomal dominant (DFNA), autosomal recessive (DFNB), X-linked (DFNX), and Y-linked (DFNY), respectively. The order in which the loci have been described is denoted by a number after these letters, e.g., DFNB1 is the first identified locus causing autosomal recessive HL [5, 6]. Earlier research reports that two-thirds of prelingual-onset sensorineural hearing loss (SNHL) is estimated to have a genetic etiology in developed countries, of which 70% is non-syndromic hearing loss (NSHL). However, 80% of NSHL is autosomal recessive non-syndromic hearing loss (ARNSHL), 20% is autosomal dominant (AD), and the remainder is composed of X-linked and mitochondrial forms [7-9]. NSHL demonstrates extreme genetic heterogeneity, with more than 55 autosomal dominant (deafness, neurosensory, DFNA), 80 autosomal recessive (deafness, neurosensory, DFNB), and 5 X-linked (deafness, neurosensory, DFNX) loci with 30, 55, and 4 causative genes, respectively, identified to date [10]. A fraction of these genes have been associated with both dominant and recessive HL. Furthermore, mitochondrial mutations can also underlie NSHL.

2.1.1. Autosomal dominant non-syndromic hearing loss

Autosomal dominant non-syndromic hearing loss (ADNSHL) is represented by heterogeneity of genetic and clinical features. ADNSHL is passed directly through generations. It is often possible to identify an autosomal dominant pattern through simple inspection of the family tree. Autosomal dominant traits usually affect males and females equally. ADNSHL associated with *GJB2* mutations is early-onset, moderate to severe, and (in contrast to autosomal recessive *GJB2* related deafness) typically progressive. Dominant *GJB2* mutations, however, often have pleiotropic effects. There is no frequent gene mutated in ADNSHL but *WFS1, KCNQ4, COCH*, and *GJB2* mutations are somewhat more frequent in comparison to the other reported genes [2, 11, 12]. Clinical manifestations and loci of known genes causing autosomal dominant non-syndromic hearing loss are summarized below in Table 1 [13, 14].

Locus Name	Gene	Onset/Decade	Audioprofile
DFNA1	*DIAPH1*	Postlingual/1st	Low frequency progressive
DFNA2	*KCNQ4*	Postlingual/2nd	High frequency progressive
DFNA2B	*GJB3*	Postlingual/4th	High frequency progressive
DFNA3	*GJB2*	Prelingual	High frequency progressive
DFNA3	*GJB6*	Prelingual	High frequency progressive

Locus Name	Gene	Onset/Decade	Audioprofile
DFNA4	*MYH14*	Postlingual	Flat/gently downsloping
DFNA5	*DFNA5*	Postlingual/1st	High frequency progressive
DFNA6/14/38	*WFS1*	Prelingual	Low frequency progressive
DFNA8/12	*TECTA*	Prelingual	Mid-frequency loss
DFNA9	*COCH*	Postlingual/2nd	High frequency progressive
DFNA10	*EYA4*	Postlingual/3rd, 4th	Flat/gently downsloping
DFNA11	*MYO7A*	Postlingual/1st	Flat/gently downsloping
DFNA13	*COL11A2*	Postlingual/2nd	Mid-frequency loss
DFNA15	*POU4F3*	Postlingual	High frequency progressive
DFNA17	*MYH9*	Postlingual	High frequency progressive
DFNA20/26	*ACTG1*	Postlingual	High frequency progressive
DFNA22	*MYO6*	Postlingual	High frequency progressive
DFNA23	*SIX1*	Prelingual	Downsloping
DFNA25	*SLC17A8*	Postlingual/2nd-6th decades	High frequency progressive
DFNA28	*GRHL2*	Postlingual	Flat/gently downsloping
DFNA36	*TMC1*	Postlingual	Flat/gently downsloping
DFNA39	*DSPP*	Postlingual	High frequency progressive
DFNA41	*P2RX2*	Postlingual	Flat progressive
DFNA44	*CCDC50*	Postlingual	Low to mild frequencies progressive
DFNA48	*MYO1A*	Postlingual	Progressive
DFNA50	*MIR96*	Postlingual/2nd	Flat progressive
DFNA51	*TJP2 & FAM189A2*	Postlingual/4th	High frequency progressive

Table 1. Clinical manifestations and locus of known genes causing ADNSHL. Adapted from [10, 14]

2.1.2. Autosomal recessive non-syndromic hearing loss

Autosomal recessive non-syndromic hearing loss at the *DFNB1* locus on chromosome 13q11-12 is characterized as congenital, typically non-progressive, mild to profound hearing impairment. The locus contains two genes, *GJB2* and *GJB6*. *GJB2* and *GJB6* are the most common mutated genes. *GJB2* is a small gene with a single coding exon. *GJB2* encodes connexin 26, a gap junction protein of the beta group with a molecular weight of 26 kd. The most common mutation is a deletion of a single guanine from a string of six *(35delG).* This mutation accounts for more than two-thirds of identified mutations and results in a frame-shift with premature termination of the protein. Profound HL caused by *GJB2* gene mutations is found in 50% of the cases; 30% are severe, 20% moderate and 1-2% are mild cases [15]. *GJB2* mutation preva-

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lence suggests that the overall prevalence of *GJB2* mutations is similar around the world, although specific mutations differ [16].

Mutations in the *GJB6* gene are the second most common genetic defect in hereditary hearing loss and lead to similar effects on abnormal expression of connexin protein Cx30. However, *GJB6* mutations are much less common than mutations in *GJB2*. In 1999, a research revealed the role for *GJB6*, i.e., adjacent to *GJB2* on chromosome 13, when a dominant mutation (T5M) was described [17]. The most common mutation in *GJB6*, however, is a >300-kb deletion that causes non-syndromic SNHL when homozygous, or when present on the opposite allele of a *GJB2* mutation [18]. *GJB6* is very similar to *GJB2* and only ~35 kb apart, but not interrupted by introns [4, 17]. Both genes are expressed in the cochlea where they can unite to form multi-unit hemichannels in the cell membrane, and function as an integral component of the potassium regulation in the inner ear. Clinical manifestations and locus of known genes implicated in autosomal recessive nonsyndromic hearing loss are summarized in Table 2 [10, 14].

Locus Name	Gene	Onset	Type
DFNB1	*GJB2*	Prelingual [1]	Usually stable
DFNB1	*GJB6*	Prelingual [1]	Usually stable
DFNB2	*MYO7A*	Prelingual, postlingual	Unspecified
DFNB3	*MYO15A*	Prelingual	Severe to profound; stable
DFNB4	*SLC26A4*	Prelingual, postlingual	Stable, progressive
DFNB6	*TMIE*	Prelinqual	Severe to profound; stable
DFNB7/11	*TMC1*	Prelinqual	Severe to profound; stable
DFNB8/10	*TMPRSS3*	Postlingual [2], prelingual	Progressive; stable
DFNB9	*OTOF*	Prelingual	Usually severe to profound; stable
DFNB12	*CDH23*	Prelingual	Severe to profound; stable
DFNB16	*STRC*	Prelingual	Severe to profound; stable
DFNB18	*USH1C*	Prelingual	Severe to profound; stable
DFNB21	*TECTA*	Prelingual	Severe to profound; stable
DFNB22	*OTOA*	Prelingual	Severe to profound; stable
DFNB23	*PCDH15*	Prelingual	Severe to profound; stable
DFNB24	*RDX*	Prelingual	Severe to profound; stable
DFNB25	*GRXCR1*	Prelingual	Moderate to profound; progressive
DFNB28	*TRIOBP*	Prelingual	Severe to profound; stable
DFNB29	*CLDN14*	Prelingual	Severe to profound; stable
DFNB30	*MYO3A*	Prelingual	Severe to profound; stable
DFNB31	*CHRN*	Prelingual	—
DFNB32/82	*GPSM2*	Prelingual	Severe to profound; stable
DFNB35	*ESRRB*	Unknown	Severe to profound
DFNB36	*ESPN*	Prelingual	—
DFNB37	*MYO6*	Prelingual	—

Locus Name	Gene	Onset	Type
DFNB39	*HGF*	Prelingual	Severe to profound; downsloping
DFNB49	*MARVELD2*	Prelingual	Moderate to profound; stable
DFNB53	*COL11A2*	Prelingual	Severe to profound; stable
DFNB59	*DFNB59*	Prelingual	Severe to profound; stable
DFNB61	*SLC26A5*	Prelingual	Severe to profound; stable
DFNB63	*LRTOMT*	Prelingual	Severe to profound; stable
DFNB67	*LHFPL5*	Prelingual	Severe to profound; stable
DFNB73	*BSND*	Prelingual	Severe to profound; stable
DFNB76	*SYNE4*	Prelingual	High frequency; progressive
DFNB77	*LOXHD1*	Postlingual	Moderate to profound; progressive
DFNB79	*TPRN*	Prelingual	Severe to profound; stable
DFNB84	*PTPRQ*	Prelingual	Moderate to profound; progressive

Table 2. Clinical manifestations and locus of known genes causing ARNSHL. Adapted from [10, 14].

2.1.3. X-Linked Non-Syndromic Hearing Loss

X-linked non-syndromic hearing loss is much rarer, accounting for 1-3% of hereditary deafness [19]. So far, there are only four genes that have been associated with X-linked non-syndromic hearing loss. These are: *PRPS1* on Xq22 that encodes phosphoribosyl pyrophosphate (PRPP) synthetase 1; *POU3F4* on Xq21, encoding a member of a transcription factor family that contains a POU domain; *SMPX* on Xp22 that encodes the small muscle protein; and *COL4A6* on Xq22 encoding the alpha-6 chain of type IV collagen. *COL4A6* is a protein-coding gene, type IV collagen having an important role in cochlea development. The *COL4A6* gene is located only ~500 kb away from the DFNX1/PRPS1 locus [20]. Clinical manifestations and locus of known genes causing X-linked nonsyndromic hearing impairment are summarized in Table 3 [10, 14].

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
DFNX6	*COL4A6*	Postlingual	Progressive Sensorinueral, mixed; mild to severe	All

Table 3. Clinical manifestations and locus of known genes causing X-linked non-syndromic hearing loss. Modified from [10, 14].

2.1.4. Non-syndromic mitochondrial hearing loss

Mitochondrial DNA (mtDNA) mutations account for at least 5% of cases of postlingual, non-syndromic hearing impairment [21]. MtDNA mutations are classified as either large-scale

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rearrangements (partial deletions or duplications) that are usually sporadic or point mutations, which are usually maternally inherited, and concern genes responsible for protein synthesis (rRNAs or tRNAs), or genes encoding subunits of the electron transport chain (ETC) [22, 23]. Tang et al. reported that mitochondrial mutations by themselves are not sufficient to produce a deafness phenotype. Modifier factors such as nuclear and mitochondrial genes, or environmental factors such as exposure to aminoglycosides, appear to modulate the phenotypic manifestations [24].

The mutation most commonly associated with maternal inheritance is A1555G on gene *12S rRNA*, *MTRNR1* [25, 26]. Non-syndromic mitochondrial hearing loss is characterized by moderate-to-profound hearing loss and a pathogenic variant in either *MTRNR1* or *MTTS1*. Pathogenic variants in *MTRNR1* can be associated with the predisposition to aminoglycoside ototoxicity and/or late-onset sensorineural hearing loss. [27].

The use of streptomycin, and to a lesser extent other aminoglycoside antibiotics, can cause hearing loss in genetically susceptible individuals. These drugs are known to exert their antibacterial effects at the level of the decoding site of the small ribosomal subunit, causing miscoding or premature termination of protein synthesis [28-30]. The hearing loss is primarily high frequency and may be unilateral. Mitochondrial non-syndromic sensory neural hearing loss (SNHL) is also associated with the A7445G, 7472insC, T7510C, and T7511C mutations in the tRNASer (UCN) gene,*MTTS1* [30, 31]. Mitochondrially inherited non-syndromic hearing loss can be caused by mutation in any one of several mitochondrial genes, including *MTRNR1, MTTS1, MTCO1, MTTH, MTND1,* and *MTTI* (Table 4).

Gene	Mutation	Possible additional symptoms
MTRNR1 (12S rRNA)	1555A->G	Aminoglycoside induced/worsened
	1494C->T	Aminoglycoside induced/worsened
	961(mutations)	Aminoglycoside induced/worsened
	827A>G	Aminoglycoside induced
MTTS1 ($_{(tRNA}$Ser(UCN)$_{)}$	7445A->G	Palmoplantar keratoderma
	7472insC	Neurological dysfunction, including ataxia, dysarthria, and myoclonus
	7510T->C	No additional symptoms
	7511T->C	No additional symptoms
MTCO1	7444G>A	Aminoglycoside associated; associated with *MTRNR1* 1555A >G
MTTH	12201T-C	No additional symptoms
MTND1	3388C-A	Tinnitus and BPPV associated
MTTI	4295A-G	Hypertrophic cardiomyopathy

Table 4. Identified mitochondrial DNA mutations in hearing loss. Modified from [10].

2.2. Syndromic hearing loss

Syndromic forms of hearing loss are less common than non-syndromic forms. To date, more than 400 genes responsible for syndromic hearing loss have been identified [32]. These can include syndromes transmitted in Mendelian or monogenic, syndromes due to chromosomal anomalies, syndromes due to multi-factorial influences, or syndromes due to a combination of these. Syndromic hearing loss can be conductive, sensorineural, or mixed.

Many of the syndromes associated with SNHL do not usually demonstrate gross inner ear anomalies. However, inner ear malformations are common in numerous syndromes. In some cases, the existence of specific inner ear anomalies may be characteristic of certain syndromes such as in BOR syndrome, Waardenburg syndrome, or X-linked deafness with stapes gusher or CHARGE syndrome. SNHL presenting later in life is often related to inner ear infections or inflammatory conditions, trauma, or tumor [33].

Mutations in the same gene may cause syndromic hearing loss in one individual and non-syndromic hearing loss in another. The most common autosomal dominant form is Waardenburg syndrome. The most common autosomal recessive forms are Pendred syndrome and Usher syndrome. Syndromic hearing loss may be transmitted as an autosomal recessive, autosomal dominant, X-linked, or matrilineal trait. Some of the genetics forms of syndromic hearing loss and their main clinical features are given in Table 5 [34].

Syndrome	Main Clinical Features	Genetics	Hearing loss
Waardenburg syndrome (AD)*	· Type 1: dystopia canthorum, iris heterochromy, brilliant blue eyes, broad nasal root, premature, graying of hair, white forelock, and vestibular dysfunction	*PAX3*	Sensorineural hearing loss
	· Type 2: similar phenotype except dystopia canthorum	MITF, SNAI2	
	· Type 3 (Klein-Waardenburg syndrome): upper extremity abnormalities other Type 1 clinical features	*PAX3*	
	· Type 4 (Waardenburg-Shah syndrome): pigmentation abnormalities and Hirschsprung's disease other Type 2 clinical features	*EDN3*, *SOX10* and *EDNRB*	
Charge syndrome (AD)	· Choanal atresia · Coloboma · Characteristic ears · Cranial nerve anomalie · Cardiovascular malformations	Mutations in *CHD7* are detected in more than 75% of CHARGE	Severe-to-profound asymmetrical mixed losses

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Syndrome	Main Clinical Features	Genetics	Hearing loss
	· Genital hypoplasia · Cleft lip/palate · Tracheoesophageal fistula · Growth deficiency · Developmental delay	patients SEMA3E	
Pierre Robin Sequence (AD)	· Micrognathia · Glossoptosis · Cleft palate	Genetic heterogeneity	Typically conductive and bilateral
Stickler syndrome (AD)	· Long and flat face · Malar and mandibular hypoplasia · Small nose with a depressed nasal bridge and anteverted nares · Altered vision · Joint problems	Mutations in *COL2A1*, *COL9A1*, COL9A2, *COL11A1*, and *COL11A2* genes	Both sensorineural and conductive
Branchio-oto-renal (BOR) syndrome (AD)	· Branchial cleft, cysts, or fistulae · Ear abnormalities · Kidney abnormalities	*EYA1*, *SIX1*, and *SIX5* mutations	Sensorineural, conductive, or mixed hearing loss
Treacher- Collins syndrome (AD)	· Zygomatic arches hypoplasia · Hypoplasia of supraorbital rims · Micrognathia · Narrow face · Antimongoloid slant of the eyes and hypertelorism · Coloboma of the lower lid with deficiency of cilia medial to the coloboma · Large nose is with hypoplastic alae · Down-turning mouth · Cleft palate · External ear abnormalities	Genetic heterogeneity: TCS-1, TCS-2 and TCS- 3 have been related to mutations in *TCOF-1*, *POLR1D* and *POLR1C* respectively	About 40-50% of patients with Treacher Collins have conductive hearing loss. Few cases of mixed hearing loss have been described.
Apert syndrome (AD)	· Craniosynostosis · Frontal bossing · Wide set eyes	*FGFR2* mutations	Mild to moderate conductive hearing loss

Syndrome	Main Clinical Features	Genetics	Hearing loss
	· Hypoplastic midface · Proptosis · Small upper jaw · Syndactyly		
Crouzon syndrome (AD)	· Synostosis · High forehead · Proptosis · External strabismus · Hypertelorism · Prognathism · Hypoplastic upper jaw	*FGFR2*	Conductive hearing loss
Saethre- Chotzen syndrome (AD)	· Brachycephaly · Low frontal hair line · Flattened nasofrontal angle · Widely spaced eyes · Ptosis · Facial asymmetry · Syndactyly · Broad or duplicated thumb or hallux	*TWIST1*	Conductive or mixed
Pfeiffer syndrome (AD)	· Broad face is midface hypoplasia · Prognatism · High forehead, flat occiput, hypertelorism · Swallowing orbits which cause proptosis · Skull malformation · Limb abnormalities	*FGFR1* & *FGFR2*	Conductive
Townes-Brock syndrome (AD)	· Anus imperforatus · Rectovaginal · Rectoperineal fistula · External ear anomalies · Thumbs malformation	It is caused by mutations in *SALL1*	Sensorineural or conductive hearing loss
Miller syndrome (AR** or AD)	· Malar hypoplasia · Micrognathia · Down-slanting eyes	*DHODH*	Conductive hearing loss- mainly due to

Syndrome	Main Clinical Features	Genetics	Hearing loss
	· Coloboma · Cleft palate · Limb defects		anomalies of middle ear
Nager syndrome (Sporadic, AD or AR)	· Limbs abnormalities · Maxillar hypoplasia · Micrognatia	Not known	Conductive or mixed hearing Loss
Goldenhar syndrome (Sporadic, AR or AD)	· Hemifacial microsomia · Auricular malformations · Vertebrae abnormalities · Facial cleft · Ocular abnormalities · Congenital heart diseases	Genetic heterogeneity	Ranges from mild to moderate conductive impairment and severe to profound sensorineural hearing loss
Usher Syndrome (AR)	· Type 1: vestibular dysfunction onset of retinitis pigmentosa in childhood · Type 2: normal vestibular response retinitis pigmentosa begins in the second decade of life · Type 3: variable vestibular response, variable onset of retinitis pigmentosa	*MYO7A, USH1C, CDH23, PCDH15,USH1G* & *CIB2, VLGR1, WHRN, PDZD7* *USH3A, CLRN1*	Profound hearing Loss Mild to moderate hearing loss Progressive hearing loss
Pendred syndrome (AR)	· Abnormal iodine metabolism (goiter)	*SLC26A4* , *FOXI1*, KCNJ10	Usually bilateral, severe to profound
Jervell & Lange-Nielsen (AR)	· SIDS, syncopal episodes prolongation of the QT interval	KCNQ1, KCNE1	Sensorineural hearing loss
Perrault syndrome (AR)	· Ovarian dysfunction in females, · Intellectual disability, · Loss of sensation and weakness in the limbs	HSD17B4, HARS2, LARS2	Sensorineural hearing loss
Alport syndrome (AR,AD, X-Linked)	· Renal abnormalities including glomerulonephritis, hematuria, and renal failure	COL4A3, COL4A4 and COL4A5	Progressive sensorineural hearing loss

Syndrome	Main Clinical Features	Genetics	Hearing loss
	· Hearing loss usually begins in the adolescent years		
Mohr-Tranebjaerg syndrome (X-Linked)	· Visual disability · Dystonia, fractures · Intellectual disability	*TIMM8A*	Progressive hearing loss
Norrie Syndrome (X-Linked)	· Eye disorder · Developmental delays in motor skills · Mild to moderate intellectual disability	NDP	Sensorineural Progressive HL

*AD- Autosomal dominant inheritance, **AR- Autosomal recessive inheritance

Table 5. Syndromic hearing loss and their clinical features. Modified from [34].

3. Genetic evaluation of hearing loss

Despite the significant advances in understanding the molecular basis of hearing loss, precise identification of genetic cause still presents some difficulties due to phenotypical variation. Gene discovery efforts for hearing disorders are complicated by the extreme heterogeneity. Usher syndrome or Jervell and Lange-Nielsen syndrome, which can be mistaken for nonsyndromic hearing loss, where Usher syndrome can be caused by mutations in several different genes. We must therefore have a clear understanding of the different types of diagnostic tests available to patients, including karyotyping, RFLP, FISH, microarray, clinical exome sequencing, preimplantation genetic diagnosis, and newborn genetic screening. Establishing a genetic diagnosis of hearing loss is a critical component of the clinical evaluation of hearing impaired persons and their families. If a genetic cause of hearing loss is determined, it is possible to provide families with prognostic information, recurrence risks, and improved habilitation options [9].

The identification of genes or genetic cause for hearing loss is a breakthrough approach. First we have to rule out non-genetic causes, then syndromic causes, and then look for non-syndromic causes. Mutations in some of these genes, such as *GJB2, MYO7A, CDH23, OTOF, SLC26A4, TMC1* are quite common for responsible of hearing loss. *GJB2* mutations are the most frequent cause of autosomal recessive non-syndromic hearing loss (ARNSHL) and account for about 20% of the cases, therefore routine screening begins with *GJB2* analysis [35]. Newborns that are diagnosed with severe-to-profound HL (in the absence of other abnormal findings on physical examination) are analyzed for mutations in the *GJB2* gene. For abnormalities such as an enlarged vestibular aqueduct indicated by imaging of the inner ear, the *SLC26A4* gene is analyzed. It is exceptional to find any gene other than *GJB2* and *SLC26A4* that is routinely analyzed in DNA diagnostics. In such cases, a positive result is only obtained in less than 20% of deaf children for which DNA diagnostics is requested [2, 36]. The key challenge lies in determining which gene is responsible in a patient with hearing loss. Sequencing all genes by

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traditional DNA sequencing technology is labor-intensive and not cost-effective [35, 36]. In such case, next-generation sequencing offers rapid sequencing of the entire human genome compared to traditional molecular testing that focuses on a single gene at a time. However, Sanger sequencing is still recommended for first-line diagnostics. It is currently the standard for molecular diagnosis of unknown point mutations in known genes. Screening can be cost-effective in individuals with genetically heterogeneous hearing loss phenotypes when a single gene is responsible for a significant percentage of cases.

3.1. Next-generation sequencing

With the fast development and wide applications of next-generation sequencing (NGS) technologies, genomic sequence information is within reach to aid the achievement of goals to decode life mysteries and improve life qualities. Today, NGS-based tests are rapidly replacing traditional tests, which include many single gene-sequencing tests for hearing loss. These tests use disease-targeted exon capture, whole-exome sequencing (WES), or whole-genome sequencing (WGS) strategies. The main advantage of these tests is that they address the problem of genetic heterogeneity, where many different genes result in phenotypes that cannot be easily distinguished clinically [36-39]. NGS also offers sequencing of very large gene or in presence of substantial locus heterogeneity, where it may be difficult to analyze the same gene by comprehensive Sanger sequencing.

NGS systems are typically represented by SOLiD/Ion Torrent PGM from Life Technologies, Genome Analyzer/HiSeq/MiSeq/NextSeq from Illumina, and GS FLX Titanium/GS Junior from Roche. Today, Illumina dominates the genome sequencing market, where instrument versatility, high throughput and accuracy, turnaround speed, faster and simpler sample preparation, and supportive data analysis software make it a driving force and the clear winner as of now. Their technology creates new applications and also decipher many existing genetic research and clinical diagnostic markets.

Targeted genomic capture and massively parallel sequencing technologies are revolutionizing genomics by making it possible to sequence complete genomes of model organisms. However, the cost and capacity required are still high, especially considering that the functional significance of intronic and intergenic noncoding DNA sequences is still largely unknown. One application that these technologies are well suited for is the re-sequencing of many selected parts of a genome, such as all exons, from a large set of genes. This requires that the targeted parts of the genome are highly enriched in the sample. Recent technological changes, such as genome capture, genome enrichment, and genome partitioning, have successfully been used to enrich large parts of the genome [40-42]. The targeted fragments can subsequently be captured using solid- or liquid-phase hybridization assays [43, 44].

Clinical exome sequencing or whole-exome sequencing is a highly complex molecular test that analyzes the exons or coding regions of thousands of genes simultaneously from a small sample of blood, using NGS techniques. Exome sequencing is especially valuable when a patient's symptoms suggest numerous possible genetic causes. The whole-exome sequencing test sequences base by base with the required depth of coverage to achieve accurate consensus sequence rather than limiting the testing to a single gene or panel of genes and incurring

diagnostic delays and escalating costs. It is possible to identify point mutations, insertions, deletions, inversions, and rearrange the exome.

Hearing status	Locus/disorder	Gene	Strategy	References
Nonsyndromic Recessive	DFNB79	*TPRN*	Targeted enrichment of genomic locus	[48]
	DFNB82	*GPSM2*	Whole exome	[49]
	DFNB84	*OTOGL*	Whole exome	[50]
Dominant	DFNA4	*CEACAM16*	Whole exome	[51]
	DFNA41	*P2RX2*	Targeted enrichment of genomic locus	[52]
X-Linked	DFNX4	SMPX	Targeted enrichment of genomic locus	[53]
Syndromic	Perrault syndrome	*HSD17B4*	Whole exome	[54]
	Perrault syndrome	*HARS2*	Targeted enrichment of genomic locus	[55]
	Carnevale, Malpuech, Michels, and oculo-skeletal-abdominal syndromes	*MASP1*	Whole exome	[56]
	Hereditary sensory and autonomic neuropathy type 1 (HSAN1) with dementia and hearing loss	*DNMT1*	Whole exome	[57]

Table 6. Deafness genes identified using genomic capture and massively parallel sequencing.

Among NGS applications, whole-exome sequencing is a cost-effective alternative to whole-genome sequencing. The total size of the human exome is ~30 Mb, which comprises ~180,000 exons that are arranged in about 22,000 genes and constitute about 2-3% of the entire human genome, but contains ~85% of known disease-causing variants. The exome refers to the portion of the human genome that contains functionally important sequences of DNA that direct the body to make proteins essential for the body to function properly. Research revealed that most of the errors that occur in DNA sequences are usually located in the exons that lead to genetic disorders. Consequently, sequencing human exome is considered to be an efficient method to discover the genetic cause of hearing disorders. Currently, sequencing whole genomes is still a substantial undertaking, which is not a routine procedure that can be done on hundreds of samples. At present, exome sequencing represents an alternative in which, approximately 30-70 Mb sequences encompassing exons and splice sites are targeted, enriched, and sequenced using commercially available sequence capture methods. Several Human Exome Sequence

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Capture kits are now commercially available. These include the Agilent SureSelect Human All Exon Kit, the Illumina Nextera Rapid Capture Exome and Nextera Rapid Capture Expanded Exome Kit, the TargetSeq In-solution Target Enrichment Kit from Life Tech/Applied Biosystems, and SeqCap EZ Exome from Roche NimbleGen. Clinical exome sequencing should be considered in the diagnostic assessment of a phenotype individual when a genetic disorder is suspected clinically, but limited genetic testing is available clinically, the patient's features are unclear or atypical, there are multiple genetic conditions as part of the differential diagnosis, and a novel or candidate gene is suspected but has yet to be discovered.

Hundreds of syndromic forms of deafness have been described, and for many of them, the underlying genes still await discovery. Since the introduction of the first NGS technology in 2004, more than 1,000 NGS-related manuscripts have been published. Until now, approximately a dozen of genes for HL have been successfully determined using NGS [45-47] (Table 6).

3.1.1. Sequencing panels

Consider a case where there is an interest in a large but limited subset of particular genes, not the whole genome, or even the whole exome, but more than just one or two genes. This sort of situation frequently arises in the context of oncology, where the characterization of a set of oncogenes on a set of pathways can help stratify cases and select the best therapeutic options. These may consist of 30-150 particular target genes, with a desire to have high throughput by analyzing multiple different specimens within a single NGS run. Generally referred to as "NGS panels," this is a third form of library which, depending on design, may start with extracting genomic DNA from a test sample where selection of targets of interest is performed. This can be by gene-specific PCR, leading to a pool of amplicons (already of the desired length, although in this case with defined endpoints), by hybridization capture, or by selective genomic DNA coding only for particular genes. This genetic material is then a very focused subset of the source genome from which to prepare the library material for dispersion and sequencing, following either of the paths above as appropriate to the sample type (note that for a direct PCR amplified genomic DNA panel type, the size shearing and adapter ligation steps may be dispensed with as these are effectively carried out in the PCR step).

A particularly clever aspect of NGS panels is that it is possible, either in the direct PCR stage for genomic DNA-based panels or in the adapter ligation step for exome-based panels, to use PCR primers or adapters, respectively, which contain an internal sequence element (commonly referred to as a "barcode") that is distinct to each sample prepared. This then allows multiple panel libraries from different samples to be mixed together prior to the dispersion and actual sequencing steps. By doing this, each individual sequence read will start with a sample-unique "barcode," allowing it to be associated back to the sample of origin. This allows many different unrelated panel sample libraries to be mixed together in one dispersion and sequencing run, thereby taking full advantage of the massively parallel nature of NGS technology and allowing for high throughput with respect to the number of samples per run. This makes panels highly cost-effective and of relatively low labor input on a per-sample basis. Depending on the type of research or clinical question being addressed in an NGS assay, the choice of the best method helps to make results cost-effective and most directly meaningful.

Different panels designed to diagnose hearing loss include:

- Hearing Loss Panel Tier 1—testing for mutations in *GJB2*, *GJB6*, *MTRNR1*, and *MTTS1* that account for 40% of the genetic causes of hearing loss. Reflex testing to OtoSeq® Hearing Loss Panel is an option for patients with normal Tier 1 results. This panel contains 23 genes, which identifies an estimated 80% of the genetic causes of hearing loss.
- OtoGenomeTM Test Panel offered by Partners Healthcare. This test panel simultaneously screens 87 genes known to cause both non-syndromic hearing loss and syndromes that can present as isolated hearing loss, such as Usher, Pendred, Jervell and Lange-Nielsen (JLNS), Branchio-Oto-Renal (BOR), Deafness and Male Infertility (DIS), Perrault, and Waardenburg syndromes.

3.1.2. NGS data analysis

The large amount of data derived from NGS platforms imposes increasing demands on statistical methods and bioinformatic tools for the analysis. Although the NGS platforms rely on different principles and differ in how the array is made, their work flows are conceptually very similar. All of them generate millions or billions of short sequencing reads simultaneously. Several layers of analysis are necessary to convert these raw sequence data into understanding of functional biology. These include alignment of sequence reads to a reference, base-calling and/or polymorphism detection, de novo assembly from paired or unpaired reads, and structural variant detection (Figure 1). To date, a variety of software tools are available for analyzing NGS data. Although tremendous progress has been achieved over the last several years in the development of computational methods for the analysis of high-throughput sequencing data, there are still many algorithmic and informatics challenges remaining. For example, even if a plethora of alignment tools have been adapted or developed for the reconstruction of full human genotypes from short reads, this task remains an extremely challenging problem. Also, when a high-throughput technology is used to sequence an individual (the donor), any genetic difference between the sequenced genomes and a reference human genome—typically the genome maintained at NCBI—is called the variant. Although this reference genome was built as a mosaic of several individuals, it is haploid, and may not contain a number of genomic segments present in other individuals. By simply mapping reads to the reference genome, it is impossible to identify these segments. Thus, de novo assembly procedures should be used instead. Nonetheless, NGS technologies continue to change the landscape of human genetics. The resulting information has both enhanced our knowledge and expanded the impact of the genome on biomedical research, medical diagnostics, and treatment, and has accelerated the pace of gene discovery [47].

Clinical diagnostic using NGS technologies may be applicable in such cases where clinicians consider a non-syndromic hearing disorder, especially after negative results on tests for mutations in the autosomal recessive *DFNB1* locus for *GJB2* or *GJB6*, according to recently published guidelines [39, 58]. Updated guidelines from the American College of Medical Genetics and Genomics (ACMG) recommend that clinicians consider NGS when testing for genetic causes of hearing loss [56]. Prior to considering genetic testing, clinicians should

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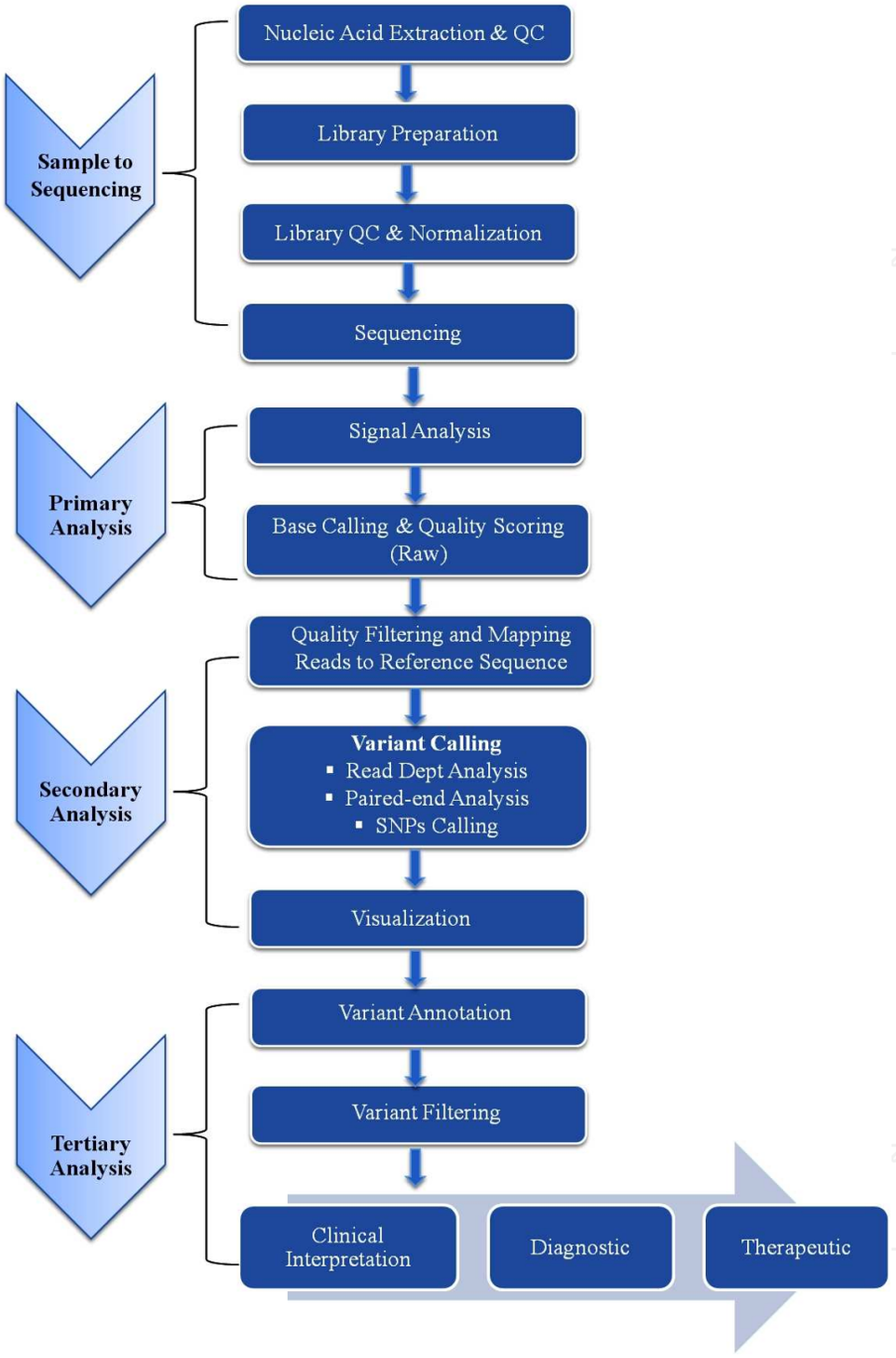

Figure 1. Next-generation sequencing: An approach from sample to analysis.

undertake a comprehensive evaluation of the patients' medical histories, including birth, that will help distinguish acquired versus inherited causes of hearing loss. They should also perform audiological evaluations to determine the type and degree of hearing loss, as recommended by ACMG. ACMG also recommends genetic testing and counseling that could include

single-gene tests, panels, genome/exome sequencing, chromosome analysis, and array-based copy number analysis if clinical findings suggest syndromic genetic hearing loss. Single-gene testing may be needed if medical and family history suggests non-syndromic hearing loss that is not associated with environmental causes. If none is suggested, the next step could be *GJB2* or *GJB6* testing. If single-gene tests yield no diagnosis, clinicians may consider NGS that quickly replaces many single-gene tests for hearing loss and can assess patients whose phenotypes are not easily distinguished clinically [58].

3.2. Cytogenetics

Cytogenetic tests are a diagnostic tool for a number of clinical syndromes associated with hearing loss. They proved the causal association between specific chromosomal abnormalities and clinical features observed in patients. Although cytogenetics is not the first technique to be considered when evaluating a child with non-syndromic deafness, this form of testing could be valuable in cases of deafness of unknown etiology, particularly if there were accompanying congenital anomalies, or a family history of multiple spontaneous abortions. When all other causes of deafness are eliminated, cytogenetics could be used to determine if the hearing loss may be due to a chromosome rearrangement, such as a balanced translocation. The advantage would be that, if such a chromosome rearrangement were found, it would immediately suggest the location of the deafness gene [59].

Cytogenetic or molecular cytogenetic tests such as karyotyping, fluorescent in situ hybridization (FISH), or chromosomal microarray analysis (CMA) may provide diagnostic information when syndromes characterized by chromosomal aneuploidies, structural rearrangements, or deletions or duplications are suspected. Genetic testing of specific individual genes (PAX3 for Waardenburg syndromes types I and III), or small panels of genes related to a specific clinical finding (FGFR-related craniosynostosis panel) may be appropriate, depending on the suspected diagnosis [60].

3.2.1. Prenatal diagnosis

Prenatal diagnosis of chromosomal aberrations requires cytogenetic analysis of amniotic fetal cells. Amniocentesis is an invasive, well-established, safe, and reliable test during pregnancy that removes a small amount of fluid from the sac around the baby to look for birth defects and chromosomal problems. Amniocentesis is done from 12 to 15 weeks of gestation for chromosomal analysis. When the amniotic sample is received in the laboratory, it is centrifuged at 750 rpm for 10 minutes. The amniotic fluid is then carefully decanted from the cell pellet into a sterile test tube, and then the cell pellet is re-suspended in amniotic fluid. Then, suitable medium supplemented with fetal bovine serum, L-glutamine, and antibiotics are added and the cultures are incubated at 37ºC in 5% CO_2 incubator. The cells are harvested at 8-10 days after culture, subjected to routine hypotonic and fixative treatments as for whole blood culture, and then the chromosomes are analyzed [61].

Genetic screening for a specific mitochondrial mutation during pregnancy could offer a strategy of minimizing hearing loss in babies from exposure to avoidable risk factors such as

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neonatal use of aminoglycoside antibiotics [3]. Genetic counseling should ideally be offered to all pregnant women who have a family history of any condition that might be tested by either amniocentesis or chorionic villus sampling (CVS). It is important to offer genetic counseling before and after prenatal diagnostic testing. The important aspects should be considered such as presentation of the background risk of congenital disease and anomaly, and individual increased risks (such as increased maternal age), options and limitations for prenatal genetic diagnosis, possible diseases that can be detected, risks associated with the relevant tests, and conflictual areas in relation to prenatal diagnosis and alternatives. [62]. Different techniques used in genetic analysis and their applications are summarized in Table 7.

Techniques	Applications	Year, Discovered/ Reported by	References
Sanger Sequencing	Sequencing of targeted genes to analyze variations	1975, Frederick Sanger	[63]
FISH (fluorescence in situ hybridization)	Detect and localize the presence or absence of specific DNA sequences on chromosomes	1980, Bauman et al.	[64]
RFLP (Restriction Fragment Length Polymorphism)	Variations in homologous DNA sequences	1984, Sir Alec Jeffreys	[65]
Microarray	Copy number variation of numbers of genes involved in disease	1995, Schena et al.	[66]
qRT-PCR	Copy number variations of targeted genes	1996, Heid et al.	[67]
SMRT (Single molecule real time sequencing)	Detects variations of entire genome and/or coding regions, genome resequencing, transcriptome profiling, DNA-protein interactions, maximum read length >40,000 bases	2003, Levene et al.	[68]
Clinical exome sequencing	Analyzes the exons or coding regions of thousands of genes simultaneously	2009, Sarah B Ng et al.	[69]

Table 7. Different techniques used in genetic analysis and applications.

Molecular genetic testing can be helpful because an etiology cannot be otherwise established in the majority of individuals with genetic hearing loss. Molecular analysis is essentially non-invasive and may reduce the need for more extensive and expensive testing; it sometimes requires sedation or general anesthesia of infants and children. Molecular analysis can be beneficial for the diagnosis of syndromic hearing loss before additional features emerge (e.g., in Pendred syndrome or Jervell and Lange-Nielsen syndrome), and can distinguish individuals with mitochondrial mutations who are at risk for iatrogenic hearing loss when treated with aminoglycosides [4]. There are other benefits of molecular analysis, which include associated knowledge of the pattern of inheritance and more accurate genetic counseling. Recently developed high-throughput techniques reduce the burden of the costs of sequencing.

For example, sequencing costs have massively reduced from $5,292.39/Mb in 2001 to $0.06/Mb by April 2013 [70]. It is estimated that the sequencing costs will further reduce with precipitous dropping per-base cost with advancing techniques.

3.3. Limitations and challenges

Despite the significant advantages of genetic testing, there are also several limitations and challenges. These limitations and challenges are briefly discussed below:

- The spectrum of DNA variation in a human genome comprises small base changes (substitutions), insertions and deletions of DNA, large genomic deletions of exons or whole genes, and rearrangements such as inversions and translocations. Traditional Sanger sequencing is restricted to the discovery of substitutions and small insertions and deletions [71].
- Although NGS promises a personalized approach to complex diseases, it has limitations. NGS cannot detect large deletions or duplications of DNA or nucleotide repeats that can cause disease. These limitations of NGS technologies may necessitate use of alternative or complementary genetic testing strategies in some cases.
- Not all regions of the genome are efficiently captured and analyzed by current exon-capture or WGS approaches, and large deletions and duplications, in addition to copy-number and structural variations, may not be efficiently detected [72].
- It is possible to determine with recent technology if an asymptomatic newborn has a mutation in the genes known to be implicated to hearing loss, although there is no certainty that all of these genes will be responsible for the incidence of hearing loss in the future.
- Current methods of DNA analysis require 2-5 mls of blood, which would be unacceptable for a newborn screen. However, It is anticipated that sufficient DNA could be extracted from a drop of blood collected for newborn bloodspot metabolic screen, with improved sequence techniques (the Guthrie test) [3].
- Genetic testing for deafness is not collectively perceived to be advantageous. Deafness is not usually considered to be negative or limiting especially by the deaf community. Many deaf individuals consider themselves to be part of their own linguistic (sign language) and cultural group, where they have their own values, identity, and traditions. It is not perceived to be a medical condition or disability. As a result, advances in hearing loss research and genetic testing might be perceived as harmful. Genetic services may be considered; however, some individuals prefer to have deaf children [4, 73].
- A positive genetic test can also lead to an increased level of anxiety and individuals may feel guilty for having potentially passed a gene alteration on to their children.

4. Development and evaluation of genetic treatment for hearing loss

Despite recent developments in medicine, there is still no cure for most types of hearing loss. The development of a biological method for the repair, regeneration, and replacement of hair

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cells of the damaged cochlea has the potential to restore normal hearing. At present, gene therapy and stem cells are two promising therapeutic applications for hearing disorders.

4.1. Gene therapy

Gene therapy involves using specific sequences of DNA to treat human disease. It is an experimental form of treatment that is still being developed, but it has a unique application for hearing loss. Two main gene therapy approaches have been considered: replacing a mutated gene that causes disorder with a healthy copy of the gene, or inactivating a mutated gene that is functioning improperly. Gene therapy technology has improved in recent years, making it a promising technique for treating hearing disorders. The gene vector, the route of gene administration, the therapeutic gene, and the target cells are four major elements of gene therapy. With the recent developments in the field, a wide variety of viral and non-viral vectors have emerged that can deliver genetic payloads to target cells in the inner ear. There are three viral vectors commonly utilized for gene therapy (targeted at the inner ear): adenoviral vectors, adeno-associated viral vectors, and lentiviral vectors. Several promising clinical trials have been reported using gene therapy.

The first study of gene therapy for hearing disorders was reported in 1994 by Fujiyoshi and colleagues. They developed the myelin basic protein (MBP) transgenic mice by microinjecting an MBP cosmid clone into the pronucleus of fertilized eggs of shiverer mice to replace the autosomal recessive mutation (deletion) gene by the transgene for MBP. The MBP transgenic mice were found to recover up to 25% of normal levels of MBP, and significantly higher myelinated axons were present in the transgenic mice compare to control mice [74, 75]. In 1996, other studies reported that foreign genes were successfully transfected into the inner ear using replication-deficient viral vectors [75-77].

The discovery of RNA interference (RNAi)-mediated gene inactivation has introduced a new mechanism for targeted therapy of the inner ear at the molecular level [78]. RNAi is an intracellular two-step process that converts precursor double-stranded RNA molecules into functional small interfering RNAs (siRNAs). Synthetic double-stranded RNAs can be introduced as siRNA mimics and used to trigger RNAi and intentionally reduce the expression of targeted genes for therapeutic applications. A few allele variants of *GJB2* cause autosomal dominant non-syndromic hearing loss as a dominant-negative consequence of expression of the mutant protein. Allele-specific gene suppression by RNAi is a potentially attractive strategy to prevent hearing loss caused by this mechanism [79]. Since inheritance is autosomal dominant, silencing the mutated allele is predicted to preserve hearing. A recent proof-of-principle study validated this prediction—an siRNA was shown to potently suppress expression of the *R75W* allele of human *GJB2* in a murine model [79, 80].

Bacterial artificial chromosome (BAC) mediated transgenesis has proven to be a highly reliable way to obtain accurate transgene expression for in vivo studies of gene expression and function. BAC transgenes direct gene expression at physiological levels with the same developmental timing and expression patterns as endogenous genes in transgenic animal models. Recently, transgene expression through the germline was demonstrated to maintain normal inner ear morphology and stable hearing function in a mouse model of human non-

syndromic deafness DFNB3 caused by a missense mutation in the *Myo15a* gene on mouse chromosome 11. 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 [80, 81].

Neurotrophic factors are essential in the development of the inner ear and are able to protect inner ear hair cells and spiral ganglion neurons from the damage caused by various pathogenic factors and promote the recovery from cochlear injury. Up to now, more than 20 neurotrophic factors have been revealed with protective effects on inner ear cells [75]. Neurotrophin gene therapy is promising both in the protection against exogenous damage and the regeneration after endogenous and exogenous damage. It has been reported that neurotrophic factors, such as BDNF (brain-derived neurotrophic factor), NT-3 (neurotrophins 3), TGF (transforming growth factor), GDNF (glial cell line-derived neurotrophic factor), FGF (fibroblast growth factor), CNTF (ciliary neurotrophic factor), and HGF (hepatocyte growth factor) have protective effects of different extents on inner ear hair cells and neurons [82-86].

The discovery of new therapies for the treatment of hereditary hearing loss will depend on a better understanding of gene function in the survival and differentiation of existing neurons, and encourages the growth and differentiation of new neurons and synapses, bonding nerves to cochlear hair cells to form synaptic connections as well as in maintaining the unique inner ear ion balance.

Atoh1 (Math1) gene acts as a "switch" to turn on hair cell growth and it is discovered that *Atoh1* is artificially switched on in the cells that support hair cells (called "supporting cells"); it instructs them to divide and form new hair cells. *Atoh1 (Math1)* plays an important role in the differentiation of hair cells of the developing inner ear and restore auditory function [87-89]. Using the tools of gene therapy may activate *Atoh1* to induce undamaged cells within the cochlea to develop into hair cells in an adult human ear and rebuild a damaged ear by replicating the steps that took place during embryonic development. There is still a lot of work to be done for human adult ear. *CGF-166* gene therapy has been shown to activate the *Atoh1* biological pathway and the gene was able to safely restore hearing in animal models. Recently, the clinical trial started to test if *CGF166* will have the same beneficial effect in humans [90]. Researchers believe that this therapy would not help people with types of inherited deafness where the structures in the ear needed to support new hair cell growth are missing or those who have damaged auditory nerves.

There is no ideal gene delivery system for gene therapy so far. Three kinds of vectors (bacterial vector, multiplex gene vector, labeled gene vector) may have great prospects. Long-term human gene therapy will not be feasible until there is substantial improvement in transduction efficiencies into human tissues. The combination of more efficient gene transfers, targeted vector systems, and effective and relatively nontoxic selection systems to maintain gene expression may make the long-term correction of hearing disorders feasible and safe. Some practices of inner ear gene therapy may need to be carried out at the embryonic stage for the treatment of hereditary hearing loss in the future.

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4.2. Stem cells therapy

The recent developments in stem cell technologies are opening novel therapeutic possibilities for the treatment of hearing disorders. Stem cell therapy is a relatively new technique used to treat many forms of human disease in which exogenous stem cells are used to replace dead or damaged endogenous cell types. In recent years, researchers have undertaken a number of successful animal studies in the area of developing stem cell therapies for hearing loss and able to turn stem cells into many of the cell types in the inner ear whose damage and death leads to hearing loss, such as hair cells and auditory nerve cells.

Stem cells are a group of cells in our bodies with the capacity to self-renew and differentiate to various types of cells, thus to construct tissues and organs. When a stem cell divides, each new cell has the potential either to remain undifferentiated (self-renewal) or become a specialized (differentiation) type of cell with a specific function. Stem cells can be classified into different types, based on their source of origin, the time of derivation, and the potential to produce different lineages. The primordial master stem cell is the zygote. The zygote and early blastomeres are totipotent and can generate any and all human cells type in the body, such as the brain, liver, blood, or heart cells. It can even give rise to a whole functional organism including extraembryonic tissues. Pluripotent stem cells have a slightly more limited potential. They have the ability to produce cell types from all three embryonic germ layers (endoderm, mesoderm, and ectoderm), including all the somatic lineages as well as germ cells; but infrequently, if ever, can produce extraembroynic lineages such as those from the placenta. It cannot form an entire functional organism. Lastly, multipotent stem cells such as hematopoietic stem cells have a more limited ability, producing cell types usually restricted to a single organ or germ layer. Multipotent stem cells have the ability to differentiate into a closely related family of cells. Pluripotent stem cells have the widest range of potential applications. They can generally be classified as embryonic or adult, depending on their developmental stage of derivation.

Embryonic and adult stem cells differ primarily in the number of different cells each can produce. Embryonic stem cells are derived from a four- or five-day-old embryo that is in the blastocyst phase of development. It can develop into any cell type of the body. In contrast, adult stem cells reside in many organs of the adult human body and can generate a range of cell types from the originating organ or even regenerate the entire original organ. Adult stem cells can be found in a great number of organs and tissues including the brain, bone marrow, peripheral blood, blood vessels, skeletal muscle, skin, teeth, heart, gut, liver, ovarian epithelium, and testis [91]. A relatively recent breakthrough in stem cell research is the discovery that specialized adult (somatic) cells can be 'reprogrammed' into cells that behave like embryonic stem cells, termed induced pluripotent stem cells (iPSCs) [92]. Like human embryonic stem cells, the iPS (induced pluripotent stem cells) cells are immortal, pluripotent, and express genes characteristic of all three embryonic germ cell layers (endoderm, ectoderm, and mesoderm) when induced to differentiate.

A number of criteria must be satisfied to achieve functional restoration, including generation of an adequate number of cells to invert the defects, differentiation of the cells to the correct phenotype, formation of appropriate three-dimensional tissue structures, and production of cells/tissues that are mechanically and structurally complaint with the native tissue without immunological rejection [80, 93]. The generation of neural stem cells and control of neural differentiation from human embryonic stem cells have opened new doors for therapy of hearing disorders. Several studies have demonstrated successful delivery of embryonic and adult stem cells to normal and damaged tissues in vivo, and in some cases a therapeutic effect has been observed.

One of the first reports of stem cell delivery to the inner ear was a study by Ito and colleagues (2001) that demonstrated survival and migration of adult rat hippocampus-derived neural stem cells (NSCs) implanted into the rat cochlea. Within 2-4 weeks of grafting to the cochlea, some NSCs survived in the cochlear cavity. Some of them had adopted the morphologies and positions of hair cells [94]. Following this study was a report about the potential of NSC transplantation to the damaged mouse cochlea. The majority of transplanted cells integrated in the vestibular sensory epithelia and expressed specific markers (myosin VIIa) for hair cells in vivo. The result of this study suggests that transplanted NSCs have the potential to differentiate and restore inner ear hair cells. However, a small number of hair cell marker-positive grafted cells and no evidence of synaptic connections between transplants and host spiral ganglion neurons hampered well-established methods for functional recovery [95]. The principal differences between human and mouse NSCs seem to be the length of the cell cycle (up to 4 days in humans) and the predilection of human cells to senesce (after ~100 cell divisions) [96]. NSCs can achieve therapeutic efficacy in human clinical applications, although many limitations remain to be overcome.

Several studies reported on the transplantation of embryonic stem (ES) cells into the inner ear. ES cells have the ability to differentiate into neuronal cell types when transplanted into the spiral ganglion of cochlea. ES cells that have been transplanted into the spiral ganglion of the cochlea were found to express neural markers [97, 98] and develop cellular processes similar to axons that extend towards the organ of Corti [99-102]. Some of these stem cell-derived neurons were shown to establish synaptic contacts with sensory hair cells, the peripheral target for spiral ganglion neurons (SGNs) in vitro (Matsumoto et al., 2008) and to survive in animals with selective loss of SGNs [99, 103].

For a cell therapy approach aiming at restoring impaired function, implanted cells need to be able to convey auditory information from the periphery to more centrally located nuclei. Recent studies have shown that dorsal root ganglion cells or ES cells are transplanted to the transected auditory nerve migrated along the nerve fibers in the internal auditory meatus and, in some cases, even reach proximate to the ventral cochlear nucleus in the brainstem [104, 105]. Interestingly, Ito et al. (2001) reported that embryonic brain tissue transplanted to the acutely transected ventral cochlear tract resulted in not only regeneration but additionally functional recovery [105, 106]. However, there are many chemical factors that produce a barrier

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between the peripheral and central nervous system and could impede the ability of central processes of replacement neurons to make a connection in the cochlear nucleus.

A number of studies have shown that adult bone marrow-derived stem cells (MSCs) can also have therapeutic potential in the damaged inner ear. MSCs have shown plasticity with a capacity to differentiate into a variety of specialized cells. MSCs have been delivered both systemically and by direct injection through the scala tympani into the mouse and gerbil cochlea respectively [107, 108]. Matsuoka and colleagues investigated the potential of MSCs to adopt properties of SGNs in vivo [108]. Identification of stem cells in the human fetal cochlea [109] contributes to the study stem cell biology of the auditory organ in humans, while advances in identification of stem cells have been made in rodents [110].

Umbilical cord blood (UCB) is the most recently identified useful source of hematopoietic stem cells (HSCs) for treatment of a wide variety of disorders. UCB has potential applications in hearing disorders. A study provided the first evidence of positive engraftment of intravenously transplanted human umbilical cord blood CD133+ HSCs into the inner ear of NOD-SCID mice rendered deaf with kanamycin and noise in vivo [111]. In another study, the researchers have demonstrated that hematopoietic stem cell transplantation (HSCT) may provide improvement in mucopolysaccharidosis-associated sensorineural hearing loss [112]. Recently, an FDA-approved clinical trial involving stem cells derived from UCB has been initiated for treatment of children with sensorineural hearing loss [113].

Mammalian cochlear hair cells do not regenerate spontaneously, although vestibular hair cells in adult mammals regenerate at levels so low as to rule out any significant functional recovery [114, 115]. The discovery of stem cells has opened the possibility of devising strategies to recruit these cells to repair damaged or lost cochlear hair cells. Stem cells are important tools for hearing disorder research and offer great potential for use in the clinic. Certain types of stem cells, such as neural stem cells, are more capable than others of replacing lost or damaged hair cells, although they have limitations. There is a great challenge in identifying more effective ways of directing stem cells to develop into inner ear hair cells. The field of auditory stem cell research is still in its infancy, although important advances are already taking place. Stem cell therapy for hearing loss is some years away from being clinically feasible.

5. Management and prevention of hereditary hearing loss

Gene therapy and stem cell treatment have still a long way to go before these treatments will be available to use in humans. Therefore, existing measures must focus on the prevention of hearing loss to decrease the frequency of genetic hearing loss. There is a need of improved implementation of genetic counseling and awareness in populations that are at high risk of hereditary hearing loss.

Early detection and intervention of hearing loss is the most important factor in minimizing the impact of hearing loss on a child's development and educational achievements. At least, all

children with a risk for hereditary hearing loss need to be given screening audiometry. The hearing loss can be progressive in nature for a person with autosomal recessive non-syndromic hearing loss caused by mutations in *SLC26A4*. In such case, audiometric testing may be warranted every year. Additionally, thyroid function should be followed if the diagnosis is consistent with Pendred syndrome [14]. Sequential audiologic examinations are essential to document the stability or progression of the hearing loss and to identify and treat superimposed hearing losses, such as middle ear effusion.

Knowledge of the genetic cause is helpful in determining the kind of damage to the auditory system that caused deafness. Identification of the underlying cause in terms of how the inner ear is damaged may assist in choosing rehabilitation strategies, such as hearing aid or cochlear implant. In children with congenital severe-to-profound autosomal recessive non-syndromic hearing loss who are positive for mutations in *GJB2* and *GJB6* at the DFNB1 locus and who elect to receive cochlear implants, performance outcome is outstanding [116]. In addition, a recent cochlear implant study stated that children with identified *GJB2* mutations, which cause an isolated insult to the cochlea without damage to the 8th nerve or the central auditory system, benefitted from cochlear implantation in the areas of speech production, speech perception, and language [4].

5.1. Genetic counseling

Genetic counseling is an important part of evaluation and management of hearing disorders. The process of genetic counseling is intended to inform patients and their families of the medical, psychological, and familial implications of genetic hearing disorders, as well as the risks, benefits, and limitations of genetic testing. In the United States, genetics professionals recommend "non-directive" counseling. It is meant to be informative and supportive rather than advise people what to do or whether or not to have children. Genetic information can help predict whether the hearing loss will remain the same or whether it will worsen over time. In addition, genetic testing can help determine if problems besides hearing loss may be present or may develop in the future. It can also help patients and families who may be at risk for conditions that can be passed down in families (inherited conditions). There are a number of people who may have quite different attitudes about deafness in their family. Some hearing parents might be concerned about having another deaf child, while others may believe that the hearing loss would not cause a problem, but they would want to know if any other associated medical problems might be involved. Likewise, deaf parents may feel comfortable about their own abilities, but would have a better opinion of not to have a deaf child, in view of the fact that other deaf parents may be more worried about the challenges of raising a hearing child [117]. In such case, the genetic counselor should be very cautious in providing information concerning the nature of the disease, the implications of being carriers (mutation carriers of genes associated with hearing loss), and the reproductive choices. Genetic counseling services for families with deafness can only be effective and appropriate if the social values of the deaf community are taken into consideration.

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6. Conclusions and future perspectives

Advances in genetic testing are already directly impacting people's lives. The demand for molecular tests is by now increasing with the discovery of the varied molecular defects underlying hearing loss. Genetic testing has now reached a stage where it is becoming increasingly applicable for precise diagnosis of hearing disorders. The development of NGS technology has made DNA sequencing not only rapid and cost-effective, but also highly accurate and reproducible. In the near future, it is expected that there will be more enhancements in the speed and cost of DNA sequencing. We are already in the modern DNA sequencing era, where aims of third- and fourth-generation DNA sequencing additionally boost the speed of sequencing and reduce costs. Although sequencing the whole genome seems exhaustive, it could be more cost-effective than having to select the genes of interest [118]. Once genome sequencing becomes more cost-effective and fast, it will accelerate the pace of gene discovery for deafness and clinical application of this discovery will be realized. Over the next few years, most molecular genetic testing will be performed on automated instruments and some genetic tests for hearing disorders will be available as at-home kits on a large scale.

One of the roles of genetic testing is to identify presently known genetic causes of hearing loss in failed hearing screening of newborns and children who are identified with childhood-onset hearing loss. Furthermore, it increases our knowledge of the genetic causes of hearing loss. The potential for increased usage of aminoglycoside antibiotics also supports the case for a genetic screening program of pregnant women for the *m.1555A G* mutation, which could avoid unnecessary cases of hearing loss. Only when a reliable estimate of the future risks of hearing loss can be made at a reasonable cost will genetic screening become viable [3].

Molecular diagnostic results should always be interpreted with caution, as our knowledge of the molecular basis of hearing loss is still evolving. Keeping pace with emerging clinical genetic technologies requires specialized genetic training as well as broad genetic literacy for patients and clinicians ordering and receiving genetics test results. Genetic information that is shared by the patient and patient's family is unique. The application of genetic tests has appropriately generated substantial debate in the community with regard to the delivery and impact of the information on clinicians, patients, and society in general. The potential for misuse of genetic information is enormous and requires action to protect the privacy of genetic information and protect individuals from discrimination based on genetic information. The ethical, legal, and social issues surrounding genetic testing for hearing loss need to be addressed. In the near future, more studies of the ethical and social aspects of genetic testing for hearing disorders should be done. It is hoped that the potential for misuse of genetic information in the future will be limited.

Some of the novel rehabilitation options under development to slow down the progression of hearing loss are gene and even mutation specific [80], suggesting that comprehensive genetic testing will be an integral part of the care of deaf and hard-of-hearing patients in the future [9]. Over time, the genetic diagnostic tests will become available faster, followed by targeted gene therapy or various permutations of progenitor cell transplantation, and eventually, the preventive interventions for a wider range of hearing impaired patients.

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