Genetic Hearing Loss and Gene Therapy

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Genetic hearing loss crosses almost all the categories of hearing loss which includes the following: conductive, sensory, and neural; syndromic and nonsyndromic; congenital, progressive, and adult onset; high-frequency, low-frequency, or mixed frequency; mild or profound, and recessive, dominant, or sex-linked. Genes play a role in almost half of all cases of hearing loss but effective treatment options are very limited. Genetic hearing loss is considered to be extremely genetically heterogeneous. The advancements in genomics have been instrumental to the identification of more than 6,000 causative variants in more than 150 genes causing hearing loss. Identification of genes for hearing impairment provides an increased insight into the normal development and function of cells in the auditory system. These defective genes will ultimately be important therapeutic targets. However, the auditory system is extremely complex which requires tremendous advances in gene therapy including gene vectors, routes of administration, and therapeutic approaches. This review summarizes and discusses recent advances in elucidating the genomics of genetic hearing loss and technologies aimed at developing a gene therapy that may become a treatment option for in the near future.

Keywords: gene therapy, genomics, hearing loss

Introduction

The World Health Organization reported that 466 million people worldwide suffers from hearing loss and estimated to rise over 900 million by 2050 [1]. Hearing loss means not able to hear as well as someone with normal hearing or a hearing threshold of more than 25 decibels in one or both ears. Hearing loss can also be classified as either conductive, sensorineural or mixed hearing loss. Conductive hearing loss is when there is a problem conducting the sound waves along the outer ear, tympanic membrane (eardrum) and ossicular chain of the middle ear towards the cochlea. Sensorineural hearing loss (SNHL) is when there is problem translating the sound vibrations into electrical signals in the sensory hair cells (HCs) inside the cochlear or damage in transmitting the information involving the afferent nerves towards the brain. This communication between the ear and brain can be damaged by aging, acoustic overexposure and ototoxic drugs. Heredity also plays a big part wherein genes for hearing are mutated or genes may increase the susceptibility to ear damage or deterioration from aging.

Hearing loss causes an annual global deficit of US $750 billion [2] which offers a high demand for an effective solution. Conductive hearing loss can be surgically managed in most patients. In contrast, SNHL is mostly irreversible and results in permanent hearing loss. However, hearing rehabilitation is possible thru hearing devices that can either be worn externally or implanted. Despite the advances in hearing aid and cochlear implant technologies, the quality of perceived sound still cannot mimic that of the normal ear. Impaired speech perception in noisy environments and musical sound perception are the biggest hurdles of cochlear implants [3, 4].

Scientist around the world are working on genomics-based research and development in hearing science. In this review, we consolidated the genes that are currently identified to be associated with hearing loss. We reviewed ways in which genes are used to restore or protect hearing and ways to deliver the genes to their target cells such as viral and non-viral vectors. We also discussed the various strategies used in gene therapy such as gene replacement, slicing and editing.
Genetic Hearing Loss

Syndromic vs. nonsyndromic hearing loss

Clinically, hearing impairment may be associated with other disorders (syndromic) or it may only be a symptom (nonsyndromic). Syndromic hearing loss occurs with malformations of the external ear, together with other malformations in other organs or organ systems. Nonsyndromic hearing loss has no associated visible deformities or the external ear or any related medical conditions, but could be associated with problems of the middle or inner ear.

Deafness genes

Genes are responsible for hearing loss among 50%–60% of children born with hearing loss [5]. According to the Hereditary Hearing Loss Homepage [6] to date, there is a total of 112 non-syndromic hearing loss genes that has been identified (Fig. 1), 71 autosomal recessive (Table 1) [7-125], 45 autosomal dominant (Table 2) [126-207], and 5 X-linked and 1 non-syndromic genes (Table 3) [208-218]. The most common cause of severe-to-profound nonsyndromic hearing loss in most populations is the autosomal recessive mutation of GJB2. While the most common cause of mild-to-moderate hearing loss is the autosomal recessive mutation on STRC [219]. On the other hand, about 30% of inherited hearing loss is associated with a syndrome [220]. Syndromic hearing impairment tends to be less genetically heterogeneous than nonsyndromic, but more than one locus has been identified for several syndromes. There are currently 11 syndromes (Table 4) [221-265] associated with hearing loss with a total of 47 syndromic hearing loss genes with 27 autosomal recessive, 13 autosomal dominant, 4 autosomal dominant or recessive and 2 X-linked recessive pattern of inheritance.

Relevance of genomics in hearing loss

With the rapid advancement of genomics, it became possible to establish high-resolution genetic and physical maps, genomic and cDNA libraries which made it easier to correlate the genes for hearing loss. The establishment of the human fetal cochlear cDNA library gave way to the cloning of majority of the genes identified related to hearing loss [266]. Screening strategies can be made in combination with next-generation sequencing platforms to study sets of deafness subjects who are likely to have the same defective gene to effectively diagnose patients with genetic hearing loss [267].

Gene therapy

As mentioned above, genetic hearing loss can now be screened in utero. In principle, gene therapy can fix a genetic mutation like the ones involving hearing genes removing or replacing the defective gene or supplying the absent gene. However, compared to other target organs for gene therapy, there are several obstacles related to the anatomy of the inner ear. The cochlea is a spiraled and fluid-filled cavity in a bony labyrinth that is very vulnerable to changes which affect the conversion of sound vibration into electrical signals. Consequently, maintaining this homeostasis is the biggest challenge in delivering any kind of therapeutic products into the inner ear. Different routes of administration have been explored with various purposes, such as efficiency in transduction and reduced cochlear toxicity. The most successful way to deliver therapeutic agents to the cochlea is an intracochlear approach through the round window membrane (RWM). The RWM is a semipermeable soft tissue separating the middle and inner ear. It allows low molecular weight molecules to up to molecules with molecular weight 45,000 under normal physiological conditions [268]. Direct injection through the RWM can also be done with a microsyringe and a narrow-gauge needle. Another option is to insert material inside the cochlear cavity to create an opening, in a procedure called a cochleostomy. This was the approach used by our group to inject material into the three cochlear cavities (scala vestibule, scala media, and scala tympani) [269, 270].

Fig. 1. Inheritance pattern of identified genes for genetic hearing loss. Drawn with data adapted from Hereditary Hearing Loss Homepage [6].
Table 1. Autosomal recessive non-syndromic hearing loss genes and loci as modified from the Hereditary Hearing Loss Homepage [6]

| Locus (OMIM)  | Location | Gene (OMIM) | Key references (PubMed) |
|---------------|----------|-------------|-------------------------|
| DFNB1A        | 13q12    | GJB2        | [7, 8]                   |
| DFNB1B        | 13q12    | GJB6        | [9]                      |
| DFNB2         | 11q13.5  | MYO7A       | [10-12]                  |
| DFNB3         | 17p11.2  | MYO15A      | [13, 14]                 |
| DFNB4         | 7q31     | SLC26A4     | [15, 16]                 |
| DFNB5         | 14q12    | Unknown     | [17]                     |
| DFNB6         | 3p14-p21 | TMIE        | [18, 19]                 |
| DFNB7/11      | 9q13-q21 | TMC1        | [20-22]                  |
| DFNB8/10      | 21q22    | TMPRSS3     | [23-25]                  |
| DFNB9         | 2p22-p23 | OTOF        | [26, 27]                 |
| DFNB10        | See DFNB8 | -               | -                       |
| DFNB11        | See DFNB7 | -               | -                       |
| DFNB12        | 10q21-q22| CDH23       | [28, 29]                 |
| DFNB13        | 7q34-36  | Unknown     | [30]                     |
| DFNB14        | 7q31     | Unknown     | [31]                     |
| DFNB15/ 72/95 | 3q21-q25,19p13 | GIPC3     | [32-34]                  |
| DFNB16        | 15q21-q22| STRC        | [35]                     |
| DFNB17        | 7q31     | Unknown     | [36]                     |
| DFNB18/18B    | 11p14-15.1| USH1C      | [37-39]                  |
| DFNB19        | 11p15.1  | OTOG        | [40]                     |
| DFNB20        | 18p11    | Unknown     | [41]                     |
| DFNB21        | 11q      | TECTA       | [43]                     |
| DFNB22        | 16p12.2  | OTOA        | [44]                     |
| DFNB23        | 10p11.2-q21 | PCDH15   | [45]                     |
| DFNB24        | 11q23    | RDX         | [46]                     |
| DFNB25        | 4p13     | GRXCR1      | [47]                     |
| DFNB26        | 4q31     | GAB1        | [48]                     |
| DFNB27        | 2q23-q31 | Unknown     | [49]                     |
| DFNB28        | 22q13    | TRIOBP      | [50, 51]                 |
| DFNB29        | 21q22    | CLDN14      | [52]                     |
| DFNB30        | 10p11.1  | MYO3A       | [53]                     |
| DFNB31        | 9q32-q34 | WHRN        | [54, 55]                 |
| DFNB32/105    | 1p13.3-22.1 | CDC14A   | [56, 57]                 |
| DFNB33        | 9q34.3   | Unknown     | [58]                     |
| DFNB35        | 14q24.1-24.3 | ESRRB   | [59, 60]                 |
| DFNB36        | 1p36.3   | ESPN        | [61]                     |
| DFNB37        | 6q13     | MYO6        | [62]                     |
| DFNB38        | 6q26-q27 | Unknown     | [63]                     |
| DFNB39        | 7q21.1   | HCF         | [64]                     |
| DFNB40        | 22q      | Unknown     | [65]                     |
| DFNB42        | 3q13.31-q22.3 | ILDRI   | [66, 67]                 |
| DFNB44        | 7p14.1-q11.22 | ADCY1   | [68, 69]                 |
| DFNB45        | 1q43-q44 | Unknown     | [70]                     |
| DFNB46        | 18p11.32-p11.31 | Unknown | [71]                     |
| DFNB47        | 2p25.1-p24.3 | Unknown | [72]                     |
| DFNB48        | 15q23-q25.1 | CIB2    | [73]                     |
| DFNB49        | 5q12.3-q14.1 | MARVELD2/BDP1 | [74-76]                |
| DFNB51        | 11p13-p12 | Unknown     | [77]                     |
| DFNB53        | 6p21.3   | COL11A2     | [78]                     |
| DFNB55        | 4q12-q13.2 | Unknown     | [79]                     |
| DFNB59        | 2q31.1-q31.3 | PIVK   | [80]                     |
### Table 1. Continued

| Locus (OMIM) | Location | Gene (OMIM) | Key references (PubMed) |
|--------------|----------|-------------|-------------------------|
| DFNB60       | 5p23.1q31.1 | SLC22A4 | [81] |
| DFNB61       | 7q22.1 | SLC26A5 | [82] |
| DFNB62       | 12p12.1q11.23 | Unknown | [83] |
| DFNB63       | 11q13.2-q13.34 | LRTOMT/COMT2 | [84, 85] |
| DFNB65       | 20q13.2-q13.32 | Unknown | [86] |
| DFNB66       | 6p2.21-22.3 | DCDC2 | [87] |
| DFNB66/67    | 6p21.31 | LFHPL5 | [88-90] |
| DFNB68       | 19p13.2 | S1PR2 | [91, 92] |
| DFNB71       | 8p22.1 | Unknown | [93] |
| DFNB72       | See DFNB15 | | |
| DFNB73       | 1p32.3 | BSND | [94] |
| DFNB74       | 12q14.2-q15 | MSRB3 | [95, 96] |
| DFNB76       | 19q13.12 | SYNE4 | [97] |
| DFNB77       | 18q12q-21 | LOXHD1 | [98] |
| DFNB79       | 9p34.3 | TPRN | [99] |
| DFNB80       | 2p16.1-p21 | Unknown | [100] |
| DFNB81       | 19p | Unknown | [34] |
| DFNB82       | 1p13.1 | (see note 4) | [101] |
| DFNB83       | See DFNA47 | | |
| DFNB84       | 12q21.2 | PTPRO/OTOGL | [102, 103] |
| DFNB85       | 17p12-q11.2 | Unknown | [101] |
| DFNB86       | 16p13.3 | TBC1D24 | [104, 105] |
| DFNB88       | 2p12-p11.2 | ELMOD3 | [106] |
| DFNB89       | 16q21-q23.2 | KARS | [107] |
| DFNB90       | 7p22.1-p15.3 | Unknown | [108] |
| DFNB91       | 6p2.5 | SERPINB6 | [109] |
| DFNB93       | 11q12.31-q13.2 | CABP2 | [110] |
| DFNB94       | - | NARS2 | [111] |
| DFNB95       | See DFNB15 | | |
| DFNB96       | 1p36.13-p36.13 | Unknown | [112] |
| DFNB97       | 7q31.2q31.31 | MET | [113] |
| DFNB98       | 21q22.3-qter | TSPEAR | [114] |
| DFNB99       | 17q12 | TMEM132E | [115] |
| DFNB100      | 5q13.2-q23.2 | PPIP5K2 | [116] |
| DFNB101      | 5q32 | GRXCR2 | [117] |
| DFNB102      | 12p12.3 | EPS8 | [118] |
| DFNB103      | 6p21.1 | CLIC5 | [119] |
| DFNB104      | 6p22.3 | FAM51B | [120] |
| DFNB105      | See DFNB32 | - | [57] |
| DFNB106      | 11p15.5 | EPS8L2 | [121] |
| DFNB108      | 1p31.3 | ROR1 | [122] |

Note 1: DFNB5 was reported originally as DFNB4.
Note 2: DFNB9 was reported originally as DFNB6.
Note 3: DFNB26 is suppressed by dominant modifier DFNM1.
Note 4: The gene at the DFNB82 locus was initially reported as GPSM2 [123], but this gene was later determined to cause Chudley-McCullough syndrome [124, 125].

### Viral vs. non-viral gene delivery

Gene transfection to inner ear cells have mostly utilized replication defective viral vectors (Table 5) [271-280]. For example, adenoviruses were used to transfer gene markers such as \( \beta \)-galactosidase and red fluorescent protein as well as functional genes such as glial-derived neurotrophic factor (GDNF) to the auditory system [270, 281, 282]. Another example is the use of adeno-associated viral vectors (AAV), such as AAV1, 2, 6, 8, and Anc80L65, which showed greater transfection efficiency in inner ear delivery [283]. Recently, the USH1 protein network component harmonin (USH1C)
Table 2. Autosomal dominant non-syndromic hearing loss genes and loci according to Hereditary Hearing Loss Homepage [6]

| Locus (OMIM) | Location | Gene (OMIM) | Key references (PubMed) |
|--------------|----------|-------------|-------------------------|
| DFNA1        | 5q31     | DIAPH1      | [126, 127]               |
| DFNA2A       | 1p34     | KCNQ4       | [129, 130]               |
| DFNA2B       | 1p35.1   | GJB3        | [132]                    |
| DFNA2C       | -        | IFNLR1      | [134]                    |
| DFNA3A       | 13q11-q12| GJB2        | [8, 135, 136]            |
| DFNA3B       | 13q12    | GJB6        | [138]                    |
| DFNA4A       | 19q13    | MYH14       | [139, 140]               |
| DFNA4B       | 19q13.32 | CEACAM16    | [142]                    |
| DFNA5        | 7p15     | GSDME       | [144, 145]               |
| DFNA6        | 4p16.3   | WFS1        | [148-151]                |
| DFNA7        | 1q21-q23 | LMX1A       | [152, 153]               |
| DFNA8        | See DFNA12 |           | -                       |
| DFNA9        | 14q12-q13| COCH        | [157, 158]               |
| DFNA10       | 6q22-q23 | EYA4        | [160, 161]               |
| DFNA11       | 11q12.3-q21 | MYO7A     | [164, 165]               |
| DFNA12       | 11q2224  | TECTA       | -                       |
| DFNA13       | 6p21     | COL11A2     | [169, 170]               |
| DFNA14       | See DFNA6 |           | -                       |
| DFNA15       | 5q31     | POU4F3      | [172]                    |
| DFNA16       | 2q24     | Unknown     | [174]                    |
| DFNA17       | 22q      | MYH9        | [176, 177]               |
| DFNA18       | 3q22     | Unknown     | [179]                    |
| DFNA19       | 10(pericentr.) | Unknown | [181]                    |
| DFNA20       | 17q25    | ACTG1       | [183-185]                |
| DFNA21       | 6p21     | Unknown     | [187]                    |
| DFNA22       | 6q13     | MYO6        | [189]                    |
| DFNA23       | 14q21-q22| SIX1        | [191, 192]               |
| DFNA24       | 4q       | Unknown     | [194]                    |
| DFNA25       | 12q21-24 | SLC17A8     | [196, 197]               |
| DFNA26       | See DFNA20 |           | -                       |
| DFNA27       | 4q12     | REST        | [199, 200]               |
| DFNA28       | 8q22     | GRHL2       | [202]                    |
| DFNA30       | 15q25-26 | Unknown     | [204]                    |
| DFNA31       | 6p21.3   | Unknown     | [206]                    |
| DFNA32       | 11p15    | Unknown     | [128]                    |
| DFNA33       | 13q34-qter | Unknown   | [131]                    |
| DFNA34       | 1q44     | NLRP3       | [133]                    |
| DFNA36       | 9q13-q21 | TMEM1      | [22]                     |
| DFNA37       | 1p21     | COL11A1     | [137]                    |
| DFNA38       | See DFNA6 |           | -                       |
| DFNA39 (see note 1) | 4q21.3 | DSPP       | [141]                    |
| DFNA40       | 16p12.2  | CRYM        | [143]                    |
| DFNA41       | 12q24-qter | P2RX2     | [146, 147]               |
| DFNA42       | 5q31.1-q32 | Unknown | [141]                    |
| DFNA43       | 2p12     | Unknown     | [154]                    |
| DFNA44       | 3q28-29  | CCDC50      | [155, 156]               |
| DFNA47       | 9p21-22  | Unknown     | [159]                    |
| DFNA48       | 12q13-q14 | MYO1A      | [162, 163]               |
| DFNA49       | 1q21-q23 | Unknown     | [166]                    |
| DFNA50       | 7q32.2   | MIHR96      | [167, 168]               |
| DFNA51       | 9q21     | TJP2        | [171]                    |
| DFNA52       | 4q28     | Unknown     | [141]                    |
Table 2. Continued

| Locus (OMIM) | Location | Gene (OMIM) | Key references (PubMed) |
|--------------|----------|-------------|-------------------------|
| DFNA53       | 14q11.2-q12 | Unknown     | [173]                   |
| DFNA54       | 5q31     | Unknown     | [175]                   |
| DFNA56       | 9q31.3-q34.3 | TNC         | [178]                   |
| DFNA57       | 19p13.2  | Unknown     | [180]                   |
| DFNA58       | 2p12-p21 | Unknown     | [182]                   |
| DFNA59       | 11p14.2-q12.3 | Unknown     | [186]                   |
| DFNA60       | 2q21.3-q24.1 | Unknown     | [188]                   |
| DFNA64       | 12q24.31-q24.32 | SMAC/DIABLO | [190]                   |
| DFNA65       | 16p13.3  | TBC1D24     | [193]                   |
| DFNA66       | 6q15-21  | CD164       | [195]                   |
| DFNA67       | 20q13.33 | OSBPL2      | [175]                   |
| DFNA68       | 15q25.2  | HOMER2      | [198]                   |
| DFNA69       | 12q21.32-q23.1 | KITLG      | [201]                   |
| DFNA70       | 3q21.3   | MCM2        | [203]                   |
| DFNA73       | 12q21.31 | PTPRQ       | [205]                   |

Note 1: Mutations in DSPP dentinogenesis imperfect associated with hearing impairment in some families.
Note 2: MYO1A has been called in to question as the causative gene for DFNA48 [207].

Table 3. Other non-syndromic hearing loss genes and loci as modified from the Hereditary Hearing Loss Homepage [6]

| Locus (OMIM) | Location | Gene (OMIM) | Key references (PubMed) |
|--------------|----------|-------------|-------------------------|
| X-linked     |          |             |                         |
| DFNX1        | Xq22     | PRPS1       | [208]                   |
| DFNX2        | Xq21.1   | POL3F4      | [209]                   |
| DFNX3        | Xp21.2   | Unknown     | [210, 211]              |
| DFNX4        | Xp22     | SMPX        | [212]                   |
| DFNX5        | Xq26.1   | AIFM1       | [213]                   |
| DFNX6        | Xp22.3   | COL4A6      | [214]                   |
| Y-linked     |          |             |                         |
| DFNY1        | Y        | Unknown     | [215]                   |
| Modifier     |          |             |                         |
| DFNM1        | 1q24     | METTL13     | [48]                    |
| DFNM2        | 8q23     | Unknown     | [216]                   |
| AUNA-Auditory Neuropathy | 13q14-21 | DIAPH3 | [217, 218] |

Note: Previous nomenclature designated X-linked loci as DFN but this has been changed to DFNX.

gene delivery using synthetic Anc80L65 vectors to treat hearing loss in mice with Usher syndrome restored complex auditory and balance behaviour similar to near wild-type levels with up to 90% transduction efficiency [276]. AAV2/8 vectors that encode wild-type whirlin (WHRN) gene restored inner hair cells (IHC) but not outer hair cells and auditory function [272]. AAV2/1 vectors were injected in transmembrane channel like 1 (TMCI) mutant mice restored moderate hearing function with minimal auditory-brainstem-response threshold [284]. A similar viral capsid and a promoter that restricted expression to IHCs partially restored auditory function in mice deficient in the IHC gene encoding for vesicular glutamate transporter 3 (VGluT3) [271]. Furthermore, the cellular tropism of a novel adeno-associated bovine virus vector efficiently transduced cochlear and vestibular HC and supporting cells without pathological effects outperforming other viral vectors [285].

The concept of gene therapy seems straightforward, but numerous problems and risks exist that prevent gene therapy using viral vectors [286]. Even with all the potential benefits of gene therapy, the utilization of viral vectors in the clinical setup is hindered by the possibility of tumorigenesis and unexpected adverse effects from virus integration in human DNA. Therefore, non-viral delivery systems are developed as an alternative to harness gene therapy. These non-viral vectors include cationic liposomes and other non-liposomal polymers along with the use of biolistic materials and electroporation (Table 6) [287-301].

Cationic liposomes are phospholipid vesicles that fuses to the cellular membrane due to their cationic charge, thereby releasing the DNA to the cytoplasm [302]. Cationic liposomes can be easily prepared in large amounts, non-infectious and has a large gene capacity. Meanwhile, synthetic and naturally occurring polycationic polymers attract negatively charged phosphates of the DNA [303]. These include polyethyleneimine, dextran, chitosan, PLGA and among others. Cationic polymers are also easy to prepare and non-immunogenic. However, both types have low transfection yields and may still provoke an acute immune response.

Another mode of gene transfection makes use of DNA-coated gold microparticles and bombarded into a targeted cellular surface by a pressure pulse of compressed
Table 4. Syndromic hearing loss genes according to Hereditary Hearing Loss Homepage [6]

| Gene (OMIM)        | Location   | Inheritance                      | Key references (PubMed) |
|--------------------|------------|----------------------------------|-------------------------|
| **Alport syndrome**|            |                                  |                         |
| COL4A3             | 2q36.3     | Autosomal recessive              | [221]                   |
| COL4A4             | 2q36.3     | Autosomal recessive              | [221]                   |
| COL4A5             | Xq22.3     | X-linked recessive               | [222]                   |
| **Branchio-Oto-Renal syndrome** |         |                                    |                         |
| EYA1               | 8q13.3     | Autosomal dominant               | [223]                   |
| SIX5               | 19q13.32   | Autosomal dominant               | [224]                   |
| SIX1               | 14q23.1    | Autosomal dominant               | [225]                   |
| **CHARGE syndrome**|            |                                    |                         |
| SEMA3E             | 7q21.11    | Autosomal dominant               | [226]                   |
| CHD7               | 8q12.2     | Autosomal dominant               | [227]                   |
| **Jervell & Lange-Nielsen syndrome** |       |                                    |                         |
| KCNQ1              | 11p15.5–15.4 | Autosomal recessive          | [228]                   |
| KCNE1              | 21q22.12   | Autosomal recessive              | [229, 230]              |
| **Norrie disease** |            | X-linked recessive               | [231, 232]              |
| NDP                | Xp11.3     | X-linked recessive               |                         |
| **Pendred syndrome** |          |                                    |                         |
| SLC26A4            | 7q22.3     | Autosomal recessive              | [233]                   |
| FOX11              | 5p35.1     | Autosomal recessive              | [234]                   |
| KCNJ10             | 1q23.2     | Autosomal recessive              | [235]                   |
| **Perrault syndrome** |          |                                    |                         |
| HSD17B4            | 5q23.1     | Autosomal recessive              | [236]                   |
| HARS2              | 5q31.3     | Autosomal recessive              | [236]                   |
| CLPP               | 19p13.3    | Autosomal recessive              | [237]                   |
| LARS2              | 3p21.31    | Autosomal recessive              | [238]                   |
| TWNK               | 10q24.21   | Autosomal recessive              | [239]                   |
| ERAL1              | 17q11.2    | Autosomal recessive              | [240]                   |
| **Stickler syndrome** |         |                                    |                         |
| COL2A1             | 12q13.11   | Autosomal dominant              | [241]                   |
| COL11A1            | 1p21       | Autosomal dominant               | [242]                   |
| COL11A2            | 6p21.32    | Autosomal recessive/dominant     | [243]                   |
| COL9A1             | 6q13       | Autosomal recessive              | [244]                   |
| COL9A2             | 1p34.2     | Autosomal recessive              | [245]                   |
| **Treacher Collins syndrome** |      |                                    |                         |
| TCOF1              | 5q32–q33.1 | Autosomal dominant              | [246]                   |
| POLR1D             | 13q12.2    | Autosomal dominant               | [247]                   |
| POLR1C             | 6p21.1     | Autosomal recessive              | [247]                   |
| **Usher syndrome** |            |                                    |                         |
| MYO7A              | 11q13.5    | Autosomal recessive              | [248]                   |
| USH1C              | 11p15.1    | Autosomal recessive              | [249]                   |
| CDH23              | 10q22.1    | Autosomal recessive              | [250]                   |
| PCDH15             | 10q21.1    | Autosomal recessive              | [251]                   |
| SANS/USH1G         | 17q25.1    | Autosomal recessive              | [252]                   |
| See Note A         | 15q25.1    | Autosomal recessive              | [253]                   |
| USH2A              | 1q41       | Autosomal recessive              | [254]                   |
| ACRV1/VLGR1/GPR98  | 5q14.3     | Autosomal recessive              | [255]                   |
| WHRN               | 9q32       | Autosomal recessive              | [256]                   |
| CLRN1              | 3q25.1     | Autosomal recessive              | [257]                   |
| **Waardenburg syndrome** |       |                                    |                         |
| PAX3               | 2q36.1     | Autosomal dominant               | [258]                   |
| MTF                | 3p13       | Autosomal dominant               | [259]                   |
| SNAI2              | 8q11       | Autosomal recessive              | [260]                   |
| SOX10              | 22q13.1    | Autosomal dominant               | [261]                   |
| PAX3               | 2q36.1     | Autosomal dominant or recessive  | [262]                   |
| EDNRB              | 13q22.3    | Autosomal dominant or recessive  | [263]                   |
| EDN3               | 20q13.32   | Autosomal dominant or recessive  | [264]                   |
| SOX10              | 22q13.1    | Autosomal dominant               | [265]                   |
Table 5. Viral vectors used in gene therapy for genetic hearing loss studies

| Viral vector               | Example                        | Load     | Animal     | Route of administration                  | Reference |
|----------------------------|--------------------------------|----------|------------|------------------------------------------|-----------|
| Adenovirus                 | Ad5-CMV-Atoh1-GFP              | Atoh1    | Guinea pig | Cochleostomy (scala media)               | [274]     |
| Ad5-CMV-Math1.11D          |                                | Math1    | Guinea pig | Cochleostomy (scala media)               | [275]     |
| Ad28-CMV-GFP + Ad28-GFAP-Atoh1 |                                | Atoh1    | Mouse      | Round window (scala tympani)            | [278]     |
| Adeno-associated virus     | AAV-mVGLUT3                     | VGLUT3   | Mouse      | Round window (scala tympani)            | [271]     |
|                            | AAV8-CMV-whirlin-GFP            | WHRN     | Mouse      | Round window (scala tympani)            | [272]     |
|                            | AAV2/Anc80L65.CMV.trunc-harm    | USH1C    | Mouse      | Round window (scala tympani)            | [276]     |
|                            | BAAV-β-actin-GFP                | β-actin  | Guinea pig | Cochleostomy (scala media)               | [279]     |
| Herpes simplex virus       | pH5V-bcl-2                      | BCL2     | Rat        | Organ of Corti explants                 | [280]     |
|                            | pH5V-BDNF-LacZ                  | BDNF     | Rat        | Spiral ganglia explant                   | [273]     |
| Lentivirus                  | Lenti-HOX-GFP                   | GFP      | Mouse      | Round window (scala tympani)            | [277]     |

Table 6. Non-viral vectors used in gene therapy for genetic hearing loss studies

| Non-viral vector           | Example                        | Load     | Animal     | Route of administration                  | Reference |
|----------------------------|--------------------------------|----------|------------|------------------------------------------|-----------|
| Cationic liposomes         | Liposomes β-gal plasmid         | β-gal plasmid | Guinea pig | RWM after cochleostomy                    | [287]     |
|                            | Liposomes eGFP plasmid          | eGFP plasmid | Mouse      | Gelfoam on RWM                           | [288-290] |
|                            | Lipofectamine 2000              | Math1    | Rat        | OC-derived cell line                     | [291]     |
| Cationic non-liposomal     | Polybrene Integrin antisense   | Integrin antisense oligonucleotide | Rat | OC-derived cell line                     | [292]     |
| polymers                   | Polybrene eGFP plasmid          | eGFP plasmid | Guinea pig | Sponge on RWM/ cochlear explants        | [293]     |
|                            | Polybrene (HPNP)                |          | Guinea pig | Scala tympani injection                  | [294]     |
|                            | Polyethyleneimine (PEI)         | eGFP plasmid | Guinea pig | Gelfoam on RWM                           | [295]     |
|                            | PLGA nanoparticles             | Fluorescent dye |          |                                          |           |
| Electroporation            | Gold particles using Gene gun   | MyoXVa   | Mouse      | OC explants                              | [296, 297]|
|                            | Electroporation                 | Math1    | Rat        | OC explants                              | [298, 299]|
|                            | Electroporation                 | Math1    | Mouse      | In utero                                 | [300, 301]|

RWM, round window membrane; eGFP, enhanced green fluorescent protein; OC, organ of Corti; PLGA, poly(lactic-co-glycolic acid).

helium gas [304]. These are not immunogenic and results in a very good in vivo activity. Electroporation is also used to create transient pores in the lipid membrane, allowing the transfection of plasmid DNA, using electric field pulses [305]. However, these methods may cause significant tissue damage during the procedure and need surgery for targeted internal organs. Gene transfer is also limited to the targeted area only.

Gene therapy strategies

Gene replacement using cDNA

Gene replacement is basically delivering a functional cDNA with the correct coding sequence to supplement a nonfunctional mutant gene of interest in specific cell types [306]. The ideal application of gene replacement is in genetic disorders caused by mutations leading to loss in phenotype, such as recessive diseases. However, effectiveness of this gene therapy is limited by the duration in which gene is delivered during development of target organs. If the mutation begins during prenatal development, gene replacement may not be able to recover normal physiology after significant malformations. In addition, an extended expression of the exogenous sequence must be maintained if the mutated gene is expressed into adulthood. Dominant deafness mutations are less likely to be recovered with gene replacement strategies but other approaches can still be utilized.

Gene silencing using RNA interference

Dominant hearing loss mutations in heterozygous animals can be “silenced” or negatively regulated by suppressing the mutant allele while allowing expression of the wild-type allele to overcome the consequences of the mutation. Gene silencing can be achieved at the transcriptional level by preventing the mRNA from being transcribed. At the post-transcriptional level, gene silencing occurs with use of RNA interference (RNAi) to prevent mRNA translation [307]. The central role in RNAi is played by two types of short complementary small RNA—microRNA (miRNA) or small interfering RNA (siRNA). In an acoustic overexposure study in mouse, siRNA was found...
to be able to silence the expression of AMP-activated protein kinase which causes HC loss and cochlear synaptopathy [308]. The main advantage of this method is its sequence specificity which makes it very suitable for silencing dominant mutations without affecting wild-type sequences or off target sequences [309].

**Gene editing using CRISPR/Cas9 system**

Another gene therapy approach that recently gained much attention to edit genome sequences is the use of the CRISPR/Cas9 system. This approach is derived from prokaryotic immune systems for resistance to phages and plasmids [310]. It is the most recent and advanced programmable nuclease adapted for genome engineering which allows for the precise direct manipulation of genome sequences in the inner ear [311]. Engineered nuclease-based enzymes are used to find a target genome sequence and to introduce single- or double-strand DNA, which stimulates innate DNA repairing machinery.

CRISPR/Cas is considered as the most pervasive and easy-to-use system with multiple applications. Cas9 requires the presence of a protospacer adjacent motif (PAM) immediately following the DNA target sequence which enables the system to be very specific but at the same time limits its clinical application [312]. To date, much effort has been directed toward the design of CRISPR nucleases with altered PAM specificities and diminished off target activities allowing even more applications [313].

**Clinical Application and Conclusions**

Gene therapy is making a comeback after safety concerns during the late 1990s and early 2000s hampered research. Gene therapy for genetic hearing loss is also getting one step closer into being a clinical treatment after several clinical trials have been approved but yet to bear results. Although gene therapy is a promising treatment option, its application is currently limited by the risk of side effects and is still under study to ensure that it will be safe and effective. In the meantime, there are 2,597 clinical trials undertaken in 38 countries that have been either completed, are in progress, or approved involving gene therapy [314]. As we wait for preliminary results to ongoing clinical trials for gene therapy for hearing loss, there are already several syndromic hearing loss genes mentioned above wherein gene therapy trials have begun for their corresponding syndromes. These include the autosomal recessive gene MYO7A causing deaf-blindness in Usher syndrome [315]. Furthermore, lessons from different approaches in gene therapy in other systems can greatly influence the advancement in design and implementation of gene therapy for genetic hearing loss. Additional advances are expected in the coming years as the field of inner gene therapy moves toward the collective goal of developing novel and effective treatments for patients with genetic hearing loss.

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**Conflicts of Interest**

No potential conflicts of interest relevant to this article was reported.

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