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REVIEW ARTICLE

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.

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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.

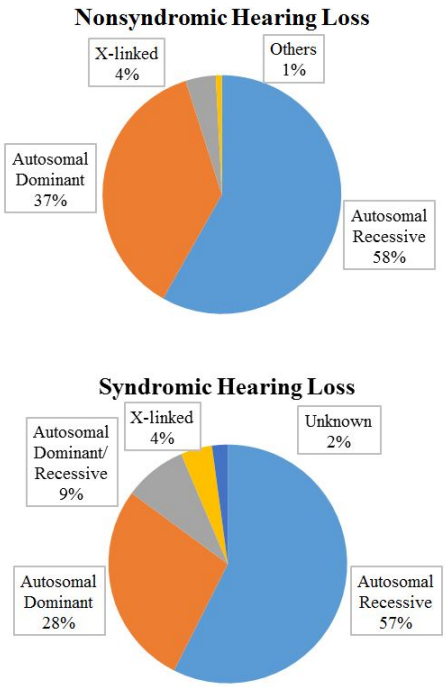

Fig. 1. Inheritance pattern of identified genes for genetic hearing loss. Drawn with data adapted from Hereditary Hearing Loss Homepage [6].

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].

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 (see note 1)	14q12	Unknown	[17]
DFNB6	3p14–p21	*TMIE*	[18, 19]
DFNB7/11	9q13–q21	*TMC1*	[20-22]
DFNB8/10	21q22	*TMPRSS3*	[23-25]
DFNB9 (see note 2)	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	11p14–15.1	*USH1C*	[37-39]
DFNB18B	11p15.1	*OTOG*	[40]
DFNB19	18p11	Unknown	[41]
DFNB20	11q25-qter	Unknown	[42]
DFNB21	11q	*TECTA*	[43]
DFNB22	16p12.2	*OTOA*	[44]
DFNB23	10p11.2–q21	*PCDH15*	[45]
DFNB24	11q23	*RDX*	[46]
DFNB25	4p13	*GRXCR1*	[47]
DFNB26 (see note 3)	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	*HGF*	[64]
DFNB40	22q	Unknown	[65]
DFNB42	3q13.31–q22.3	*ILDR1*	[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	*PJVK*	[80]

Table 1. Continued

Locus (OMIM)	Location	Gene (OMIM)	Key references (PubMed)
DFNB60	5q23.2–q31.1	*SLC22A4*	[81]
DFNB61	7q22.1	*SLC26A5*	[82]
DFNB62	12p13.2–p11.23	Unknown	[83]
DFNB63	11q13.2–q13.4	*LRTOMT/COMT2*	[84, 85]
DFNB65	20q13.2–q13.32	Unknown	[86]
DFNB66	6p21.2–22.3	*DCDC2*	[87]
DFNB66/67	6p21.31	*LHFPL5*	[88-90]
DFNB68	19p13.2	*S1PR2*	[91, 92]
DFNB71	8p2221.3	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	9q34.3	*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	*PTPRQ/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	6p25	*SERPINB6*	[109]
DFNB93	11q12.311–q13.2	*CABP2*	[110]
DFNB94	-	*NARS2*	[111]
DFNB95	See DFNB15	-	-
DFNB96	1p36.31–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	*FAM65B*	[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 β-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	*TMC1*	[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	*MIRN96*	[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[a]	Xq22	*PRPS1*	[208]
DFNX2	Xq21.1	*POU3F4*	[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			[217, 218]
AUNA1	13q14-21	*DIAPH3*	

[a]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 (*TMC1*) 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 polyethylenimine, 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			
KNCQ1	11p15.5–15.4	Autosomal recessive	[228]
KCNE1	21q22.12	Autosomal recessive	[229, 230]
Norrie disease			
NDP	Xp11.3	X-linked recessive	[231, 232]
Pendred syndrome			
SLC26A4	7q22.3	Autosomal recessive	[233]
FOXI1	5q35.1	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]
ADGRV1/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]
MITF	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	Rat	Cochleostomy (scala media)	[279]
Herpes simplex virus	pHSV-blc-2	BCL2	Rat	Organ of Corti explants	[280]
	pHSV-BDNF-LacZ	BDNF	Rat	Spiral ganglia explant	[273]
Lentivirus	Lenti-HOX-GFP Lenti-WOX-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	Guinea pig	RWM after cochleostomy	[287]
	Liposomes	eGFP plasmid	Mouse	Gelfoam on RWM	[288-290]
	Lipofectamine 2000	Math1	Rat	OC-derived cell line	[291]
Cationic non-liposomal polymers	Polybrene	Integrin antisense oligonucleotide	Rat	OC-derived cell line	[292]
	Dendritic polymers (HPNP)	eGFP plasmid	Rat	Sponge on RWM/ cochlear explants	[293]
	Polyethylenimine (PEI)	eGFP plasmid	Guinea pig	*Scala tympani* injection	[294]
	PLGA nanoparticles	Fluorescent dye (Rhodamine)	Guinea pig	Gelfoam on RWM	[295]
Biolistic	Gold particles using Gene gun	MyoXVa	Mouse	OC explants	[296, 297]
Electroporation	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, effectivity 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 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 CRISPE/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 stimulate innate DNA repairing machinery.

CRISPR/Cas is considered as the most pervasive and easy-to-use system with multiple applications. Cas9 require 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 CRIPSR 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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