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Genetics Of Hearing Loss: Focusing On Gene Therapy

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ABSTRACT

Genetic is one of the most common causes of hearing loss with more than a hundred genes related to hearing loss have been identified. A genetic diagnosis can assist clinicians to keep away from unimportant and expensive clinical examinations, put up prognostic information, and assist in constructing medical therapy for patients. The development of gene therapy to improve hereditary hearing loss continues to grow these days, showing promising results. This literature review aims to review the genetic aspects of hearing loss, focusing on the gene therapy.

1. Introduction

Most causes of hearing loss are genetic. Until now, more than 100 genes related to hearing loss have been identified.[1,2] This number continues to rise because of advances in genetic testing.[3] Based on its association with other clinical problems, hearing loss can further be classified into non-syndromic and syndromic hearing loss. Non-syndromic hearing loss predominant in most inherited hearing loss with more than seventy loci have been identified as shown in Table 1. There are also more than four hundred syndromes that have been found to be related to hearing loss with Pendred Syndrome becoming the most frequent etiology. Examples of syndromic hearing loss are shown in Table 2.[4] There can be more than one gene that is involved in syndromic hearing loss. For example, genes involved in Waardenburg syndrome are PAX3, EDN3, MITF, SOX10, and EDNRB.[5]

Figure 1 below reveals the algorithm diagnosis of inherited hearing loss. Detailed history taking, physical examination, and audiometry examination are the mainstay of initial assessment for patients with hearing loss. Three-generation pedigree and family medical history are also important data that can be retrieved through direct evaluation or medical records. Imaging, electrocardiogram, and ophthalmological evaluation are additional examinations that may be indicated in selected cases. If acquired hearing loss is presumed, then another testing can be done as indicated such as meningitis, rubella, or cytomegalovirus (CMV) testing. If syndromic hearing loss is suspected, clinicians can initiate targeted gene testing based on the presumed diagnosis. Single-gene tests such as SLC26A4 and mtDNA 1555A>G are considered in non-syndromic hearing such as, but targeted panel tests also can be done based on physical diagnosis and additional examinations.[1]

Because of highly heterogenous genetic causes of hearing loss, further studies on these genes can enhance the comprehension of the inner ear role at the molecular level.[6] A genetic diagnosis can assist clinicians to keep away from unimportant and expensive clinical examinations, put up prognostic information, and assist in constructing medical therapy for patients.[7] The development of gene therapy to improve hearing loss continues to

grow. This literature review aims to review the important features of gene therapy for genetic hearing loss.[8]

Table 1. Genes involved in Non-Syndromic hearing loss.[4]

Non-Syndromic Hearing Loss	
Pattern of Inheritance	**Genes Involved**
Autosomal Dominant	More than 25 genes have been related to this type, such as ACTG1, CCDC50, CD164, CEACAM16, KCNQ4, etc.
Autosomal Recessive	Seventy genes have been associated with this type, such as ADCY1, BDP1, BSND, CABP2, GIPC3, GPSM2, GRXCR1, GRXCR2, ROR1, etc
X-linked	Genes involved in this type are PRPS1, SMPX, POU3F4, COL4A6, and AIFM1
Mitochondrial	Genes implicated in this type are MT-TS1, MT-RNR1, and MT-CO1

Table 2. Examples of syndromic hearing loss.[4]

Syndromic Hearing Loss	
Pattern of Inheritance	**Syndrome**
Autosomal Dominant	• Waardenburg syndrome • Brachiootorenal spectrum disorders • Neurofibromatosis
Autosomal Recessive	• Stickler syndrome • Usher syndrome • Pendred syndrome • Jervell and Lange-Nielsen syndrome • Biotinidase deficiency • Refsum disease
X-linked	• Alport syndrome • Mohr-Tranebjaerg Syndrome (MTS) • X-linked Deafness-dystonia-Optic neuropathy (DDON)
Mitochondrial	• Mitochondrial encephalopathy • Lactic acidosis • Stroke-like episodes (MELAS) • Myoclonic Epilepsy Associated with Ragged Red Fibers (MERRF) • Kearns-Sayre syndrome

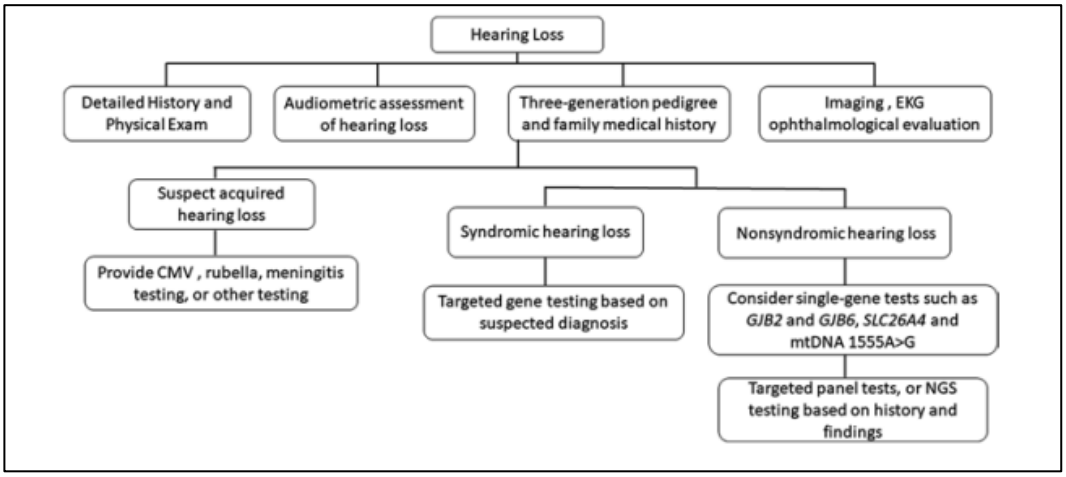

Figure 1. Algorithm Diagnosis of Hearing Loss.[1]

2. Gene Therapy for Genetic Hearing Loss

As one of the management modalities in genetic disease, including hereditary hearing loss, gene therapy works by introducing specific cell function-modifying genetic substance into a human. Gene therapy as the management for hearing loss keeps developing, especially in animal models, but it still needs time for a successful transition from animal models to humans.[9] The important aspects in understanding gene therapy are vectors, routes of delivery, targets, and its strategy.

Vectors

In gene therapy, the vector is analogous to the vehicle for the transportation of genetic substances in numerous organs and tissues. Vectors can be divided into two categories: non-viral and viral vectors. These vectors are either single-stranded DNA or double-stranded DNA. Many vectors have been developed, but most of these vectors are inadequate for replication and give rise to little menaces of viral-induced disease.[10] They also have scanted transportation capability and some of them have short-expression duration.[6] Adeno-associated viruses (AAV), Adenoviruses (AdV), and Lentiviruses (HIV-1, FIV) are some of the frequent vectors that have been evaluated in the internal ear.[9]

Adeno-associated viruses (AAV)

Of all these vectors mentioned above, AAV is the most attractive for cochlear gene transportation. AAV are tiny virus (approximately 25 nm) that belongs to the genus Dependovirus and the Parvoviridae family. Its immunogenicity and pathogenicity are minimal, and it also can transduce either dividing or non-dividing cells to give durable and steady-state gene expression.[11] The significant limitation of AAV is AAV has a little capacity (approximately 7.7 kb), and using a self-complementary version will half the capacity.[12] But, dual injection techniques can overwhelm this limitation at the cost of transduction effectiveness. This technique has been done to carry *Otof* (approximately 6 kb) into the mice's inner ear which is deficient in this gene.[13]

Some new synthetic types have been developed to be another choice for conventional AAV vectors and these vectors have shown their better capability in transduction in the inner ear.[7] Anc80L65, an efficient representation of the combinatorial library of AAV evolutionary ancestors (Anc80Lib), becomes the most widely used synthetic vector.[14] Anc80L65 showed extraordinary transduction capability in murine liver, retina, muscle tissues, and also inner ear cells.[15,16] No harmful effects were observed on vestibular function, sensorineural function, Auditory brainstem response (ABR), and Distortion product otoacoustic emissions (DPOAE). [17] Therefore, Anc80L65 is an appropriate vector for transportation of the inner ear gene, it is not only for outer hair cells (OHCs) and inner ear cells (IHCs) but also for other IHCs types.[18]

Exosome-Associated AAV (Exo-AAV) is developed by connecting the exosomes with the virus to expand the transduction capability of traditional AAV. In mice models with hereditary hearing loss, Exo-AAV gene therapy can partly improve hearing and equilibrium-related abnormal motion.[9] Although the exosomes are powerful couriers of AAV for transportation to internal ear cells, their side effects should be considered.[7]

Adenoviruses (AdV)

Adenoviruses (AdV) belong to the *Adenoviridae* family. It is well known that more than fifty serotypes of adenoviruses can infect humans, but some serotypes can be utilized for gene transfer. Those serotypes are AdV-2 and AdV-5. Adenoviruses are good gene transfer vectors for gene therapy in the internal ear because of their ability to infect numerous cell types (both non-dividing and dividing). The third generation of adenoviruses also give high-level and probably long-term expression. Limitations of adenoviruses are their first and second generations can instigate significant immune response and their limited efficacy in hair cells.[9]

Lentivectors (LV)

Lentiviruses, RNA viruses that belong to the *Retroviridae* family, are based on feline immunodeficiency virus (FIV) or human immunodeficiency virus (HIV) type 1. They are commonly pseudotyped which means that cell tropism is increased by merging vector with external viral envelope proteins. Advantages of this vector are its capability to transduce either non-dividing or dividing cells, and the ability to self-inactivate and give stable transgene expression in non-dividing cells. But, this vector has limited capability in the transduction of inner and outer hair cells and some probable ototoxic effects.[9]

Nonviral Vectors

Nonliposomic polymers, dendrimers, and cationic liposomes are some nonviral transport vectors that have been utilized in gene therapy for hearing loss. They can be made in nonbiological conditions. They are also nonimmunogenic and biologically safe. Even though these techniques are not largely implemented, they can be an

appropriate alternative and can be probably better than viral vectors in selected cases.[9]

Routes of Delivery

The preferred target in gene therapy is the inner ear because the inner ear is relatively reachable.[9] Systemic distribution is limited because there are large diffusion and physical barriers such as the labyrinthine-blood barrier. Even though this structure is not as compact as the blood-brain barrier (BBB), this barrier can delay the distribution of large substances like viral vectors and other reagents, thus restricting the efficacy of systemic transportation of therapeutic substances. These properties are important to ensure that substances administered into the cochlea will stay inside the target organ. It can make high concentrations can be kept in the target organ while distribution and toxicity are minimized.[9] Therefore, direct and local infiltration of vectors (viral) into the internal ear is preferred. Accepted injection techniques are ranging from canalostomy, cochleostomy, round window membrane (RMW) infiltration, and combination of round window membrane with canal fenestration (CF).[13]

Targets

Hereditary hearing loss is caused by mutations that influence the functions of numerous different internal ear cell types. A detailed comprehension of the physiology of every cell type is needed for the better development of proper intervention which targets different cell types. Hair cells, Stria vascularis (SV), spiral ganglion neurons (SGN), and supporting cells are several inner ear cell types that are targeted by gene therapy.[9]

Compared to other inner ear cell types, sensory hair cells have become a pivot of gene therapy research because more than half of mutations in hearing loss are expressed in hair cells. The hair bundle, a structure with an important role in the transduction of the auditory mechanoelectrical process, is the most frequently influenced part of the hair cell.[9]

Strategies

The method for gene therapy is divided into gene-specific and non-specific. Gene-specific strategies consist of gene replacement, suppression of genes, and genome editing. An example of a non-specific strategy is cell replacement (stem cell-based therapy).[9]

Gene replacement involves transporting an appropriate functional cDNA's coding sequence for a target gene into selected cell types to augment a nonfunctional mutant gene.[23] Therefore, the disease caused by loss of function mutations is perfect for this type of gene therapy. Gene silencing is utilized to 'shut down a mutant gene's expression.[24] This method can be helpful for dominant mutations. Gene editing is performed by directly manipulating the inner ear's genome sequences.[9,25]

Two main methods of stem-cell-based therapies are inner ear stem cell induction and cell transplantation. Inner ear stem cell induction can be done by two submethods. Compared to the cochlear hair cells of mammals, the cochlear hair cells of avians do not lack the regenerative capability, and therefore supporting cells' mitosis can replace these cells after acoustic trauma. Using progenitor cells to regenerate hair cells has shown trans-differentiation of supporting cells of the inner ear into hair cells and supporting cells' mitosis. The success of both submethods depends on the supporting cells' environment, with the help of intracochlear medication delivery to achieve direct results.[9] Cell transplantation is an alternative to inner ear stem cell induction. Until now, adult-derived stem cells (ASC), induced pluripotent stem cells (iPSC), and embryonic stem cells (ESCs) are some of the seeds that have been used to generate hair cells.[23] Compared to the other seeds, the embryonic type have the superiority of their pluripotency and can be differentiated into otic sensory neurons, SGN, and hair cell-like cells. But, this cell has a limited available pool. Adult-derived stem cells have limited pluripotent capability, therefore, most researchers focus on studying induced pluripotent stem cells.[24,25]

3. Conclusion

The fast progress of gene therapy development in hereditary hearing loss has been done lately. Many studies have reported positive results about gene therapy in animal models with hearing loss. Therefore, it is important to have a great comprehension of various aspects of hearing loss' gene therapy.

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