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REVIEW

Genetic Effects on Sensorineural Hearing Loss and Evidence-based Treatment for Sensorineural Hearing Loss

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Abstract In this article, the mechanism of inheritance behind inherited hearing loss and genetic susceptibility

in noise-induced hearing loss are reviewed. Conventional treatments for sensorineural hearing loss (SNHL), i.e. hearing aid and cochlear implant, are effective for some cases, but not without limitations. For example, they provide little benefit for patients of profound SNHL or neural hearing loss, especially when the hearing loss is in poor dynamic range and with low frequency resolution. We emphasize the most recent evidence-based treatment in this field, which includes gene therapy and allotransplantation of stem cells. Their promising results have shown that they might be options of treatment for profound SNHL and neural hearing loss. Although some treatments are still at the experimental stage, it is helpful to be aware of the novel therapies and endeavour to explore the feasibility of their clinical application.

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HEARING loss always involves internal and external factors, in which internal factors may be related to genetic background. For example, after exposure to the same noise, the levels of noise-induced hearing loss (NIHL) varies among people, with the variability up to 50%, which might be attributable to genetic susceptibility.^{1, 2} NIHL is one kind of sensorineural hearing loss (SNHL), and SNHL can be divided into two classes, i.e. congenital and acquired.

Congenital deafness is a hearing loss which presents at birth, or a hearing loss that may develop afterwards, but due to the factors that affect the hearing system in fetus. There are two kinds of congenital deafness, non-inherited hearing loss (20%), e.g. association with rubella infection,^{3, 4} and inherited hearing loss (80%).^{5, 6} Obviously, genetic factors play important roles in the inherited hearing loss.

INHERITED SENSORINEURAL HEARING LOSS

Inherited congenital hearing loss can be divided into four classes, autosomal recessive (74%), autosomal dominant (24%), X-linked (1%-2%), and mitochondrial gene-related.⁷ In each class, if hearing loss is the only manifestation with no other concomitant medical findings, it is called non-syndromic hearing loss. If hearing loss is accompanied with a group of signs and symptoms, this might indicate syndromic deafness.⁸

Autosomal recessive deafness

Autosomal recessive non-syndromic deafness

Autosomal recessive non-syndromic deafness is characterized by pre-lingual onset of SNHL, which is usually non-progressive, and without other medical findings at the same time. Two mutant alleles are required for the development of this deafness, one from each parent, and the inheritance follows Mendelian's law.⁹ About 37 genes have been identified

related to autosomal recessive non-syndromic deafness,¹⁰ 50% of the patients have mutations in GJB2 gene.¹¹⁻¹³

Autosomal recessive syndromic deafness

Usher syndrome is the most common form, characterized by dual sensory impairments. Both sensory hair cells (hearing) and photoreceptor cells (vision) are affected, so the patients have both congenital SNHL and progressive retinitis pigmentosa. The most frequent mutation in Usher syndrome is in USH2A gene.^{14, 15}

Pendred syndrome is the second common form of autosomal recessive syndromic deafness, which is characterized by congenital SNHL and euthyroid goiter in early puberty. About 50% of the patients have mutations in SLC26A4 gene.¹⁶

Lange-Nielsen syndrome is the third common form of autosomal recessive syndromic deafness. Besides SNHL, it is associated with cardiac arrhythmias. KCNQ1 gene mutations have been found in this syndrome, which encodes KvLQT1, a voltage-gated potassium channel protein.^{17, 18}

Goldenhar's syndrome is a complex of SNHL and oculoauriculovertebral dysplasia, which are attributed to diversified genetic loci.¹⁹

Autosomal dominant deafness

Autosomal dominant non-syndromic deafness

In autosomal dominant, a pathogenic gene is passed directly through generations, called vertical pattern of inheritance, which also follows Mendelian's law.²⁰ Autosomal dominant deafness is characterized by post-lingual progressive SNHL, while in autosomal recessive deafness the SNHL is usually non-progressive. 27 genes have been identified as the causes of autosomal dominant nonsyndromic hearing loss.^{9, 20, 21}

Autosomal dominant syndromic deafness

Waardenburg syndrome (WS) is the most common form,

which is characterized by SNHL and abnormal tyrosine metabolism (pigmentary disorder). Four types have been identified, i.e. WS I-WS IV. WS I and WS III are caused by PAX3 gene mutation; some WS II are caused by MITF gene mutation; and mutations in EDNRB, EDN3, and SOX10 genes are involved in WS IV.²²

Branchio-oto-renal syndrome is the second common form, characterized by conductive, sensorineural, or mixed hearing loss, together with branchial cleft cysts/fistulae and renal anomalies. Mutations of EYA1 gene can be identified in some cases;²³ mutations of SIX1 gene²⁴ and SIX5 gene²⁵ were identified in other cases.

Stickler syndrome (STL) is characterized by progressive SNHL and osteoarthritis. Three types have been identified, STL1 (COL2A1 gene), STL2 (COL11A1 gene), and STL3 (COL11A2 gene). The mutant COL2A1 and COL11A1 genes can express in the eyes, resulting in progressive myopia and retinal detachment, but COL11A2 gene is not expressed in the eye.²⁶

Neurofibromatosis type 2 (NF-2) is characterized by multiple central neurofibromatosis. Hearing loss is usually secondary to the growth of a vestibular schwannoma. Mutations of NF-2 gene on Chr 22q12 are responsible for this disease.²⁷

X-linked hearing loss

Inherited congenital hearing loss can be X-linked, which can be either recessive or dominant. X-linked dominant inheritance means that only one copy of the alleles is sufficient to cause hearing loss, but it is less common. In X-linked recessive, on the other hand, a male will be affected if the abnormal gene is present on his X chromosome; but in female, hearing loss will only occur in homozygous individuals, while a heterozygous female, having only one copy of the mutated allele, is a carrier rather than a patient.²⁸

However, penetrance varies significantly in X-linked inheritance due to many reasons, which are not clarified yet; sometimes it is hard to define recessive or dominant.²⁹

X-linked non-syndromic hearing loss

In X-linked non-syndromic hearing loss (DFNX), four loci (DFN2, DFN3, DFN4, and DFN6) had been previously detected, but a new classification (DFNX1-5) has been proposed. PRPS1 gene, located on Xq22, is responsible for DFNX1 (DFN2).³⁰ POU3F4 gene mutation can cause DFNX2 (DFN3), located on Xq21.1.³¹ The gene causing DFNX3 (DFN4) is unknown, but the location of mutation is identified on Xp21.2.³² SMPX gene mutation, on Xp22, is responsible for DFNX4 (DFN6).³³ The gene which can cause

DFNX5 (AUNX) is unknown, but the location of mutation is found on Xq23-q27.3.³⁴

X-linked syndromic hearing loss

Alport syndrome is caused by mutations in COL4A3, COL4A4, or COL4A5 gene. The classic phenotypes are progressive SNHL and concomitant renal failure. About 85% of Alport syndrome are X-linked inheritance.³⁵

Mohr-Tranebjaerg syndrome is an X-linked recessive inheritance. Male patients are characterized by SNHL which develops in early childhood followed by progressive dystonia, ataxia, etc. Female carrier may have mild hearing impairment and focal dystonia. This syndrome is caused by TIMM8A gene mutation, which results in mitochondrial dysfunction.³⁶

Mitochondrial gene-related hearing loss

Mutations of mitochondrial gene have been involved in many diseases, and the inheritance follows maternal line.³⁷ The mutations of mitochondrial gene, MT-RNR1 and MT-TS1, can cause non-syndromic hearing loss, but the mechanism is unknown yet.³⁸

Mitochondrial syndromic hearing loss is also common. For example, in Japan, 61% of the diabetic patients carrying one single base mutation of MTTL1 gene (3243 A-to-G transition), a mitochondrial gene, would develop SNHL, and this hearing loss would develop only after the onset of the diabetes mellitus.⁹

GENETIC FACTORS ON ACQUIRED SENSORINEURAL HEARING LOSS

Inherited hearing loss is less common than acquired SNHL.⁷ Some acquired SNHL are preventable and treatable if early diagnosis is available. It is important to understand the effect of genetic factors on acquired SNHL, so as to identify people at high risk and protect them from noise exposure.

One common acquired SNHL is presbycusis, which appears to be a multifactorial disorder involving genetic and environmental factors.³⁹ A clear familial aggregation has been found in presbycusis, supporting a genetic effect on the inheritance of presbycusis.⁴⁰ Individuals who carry two mutations in the hearing loss-associated gene GJB2 possibly have an increased risk of developing early presbycusis.⁴¹

Another acquired SNHL is noise-induced hearing loss (NIHL). Noise exposure can induce cochlear damage. Noise vibration might cause mechanical destruction of hair cells, basilar membranes and other supporting structures.⁴²⁻⁴⁸ Noise stimulation could increase the formation of

mitochondrial free radical in the cochlear, which might lead to hearing loss,⁴⁹⁻⁵⁴ and DNA is particularly susceptible to the damage mediated by free radical.⁵⁵ In addition, noise exposure could reduce blood supply to inner ear.⁵⁶⁻⁶⁴ The formation of free radical and the reduction of blood supply might co-exist, the synergism effect between them could cause excitotoxic neural swelling, necrotic and apoptotic cell death.⁶⁵

However, the development of NIHL is a complicated process. Generally speaking, a person with susceptibility genes is considered to be vulnerable to NIHL, and prone to develop NIHL if exposed to excessive noise. In a word, the interaction between genetic susceptibility and noise exposure will cause NIHL. This review focuses on the aspect of genetic susceptibility in NIHL.

Animal study

Some genes have been identified in mice which can contribute to susceptibility to NIHL. In C57BL/6J mice, age-related hearing loss locus 1 (Ahl1) was associated with the susceptibility to NIHL.^{66, 67} Cdh23 which encodes cadherin23 was identified as one of the responsible genes at the Ahl1 locus.⁶⁸ Mice heterozygous for a null allele of Cdh23 were more susceptible to NIHL compared with their wild-type littermates.⁶⁹

Human study

Some susceptibility genes for NIHL have been identified in human. They can be classified into several groups, such as CAT, an oxidative stress gene which encodes catalase,⁷⁰ KCNQ4 and KCNE1 genes which encode potassium ion channels,^{71, 72} and HSP70 genes which encode heat shock protein.^{1, 2}

CAT gene

In two independent samples, a significant association was found between CAT gene and NIHL, so CAT is confirmed as a susceptibility gene to NIHL, but CAT can take effect only when the individual is exposed to noise. This indicates the complex interplay between genetic background and environmental factor in the development of NIHL.⁷⁰

KCNE1 and KCNQ4 gene

KCNE1 and KCNQ4 genes can regulate potassium recycling pathway, mutations in these genes can cause both syndromic and non-syndromic hearing loss, indicating that these genes are indispensable for hearing function.⁷¹⁻⁷⁴

HSP70 gene

HSP70 encodes heat shock proteins which are expressed

both under physiological and pathological circumstances. In cochlear, the expression of heat shock proteins can be induced by acoustic overstimulation.^{1, 2, 75, 76}

In Chinese NIHL patients, three single nucleotide polymorphisms (SNPs) were genotyped in three HSP70 genes respectively.¹ The same three SNPs were also detected in other NIHL patients in Sweden and Poland, with significant association found between the three SNPs and susceptibility to NIHL.² The consistency among the three independent population samples indicates that the HSP70 genes may be true NIHL susceptibility genes.

About 27 genes have been identified which are related to NIHL, but the information is still very limited about the detail of how the genetic factors can influence NIHL. No formal study about the inheritance is available for the simple reason that it is very hard to find the twins or families in which all the subjects have been exposed to equal noise exposure.^{72, 77}

TREATMENT FOR SENSORINEURAL HEARING LOSS

Obviously, hearing aid and cochlear implant can provide assistance to improve hearing function. However, these treatments have their limitations. Hearing aid does not work well for severe hearing loss. Cochlear implant, on the other hand, will not provide normal hearing, and it takes a while for people to get used to the sounds provided by cochlear implant. Cochlear implant cannot help all the patients of profound hearing loss. In addition, the performance of cochlear implant varies, in some cases, it does not help to improve intelligible speech.⁷⁸

For NIHL, hearing aid or cochlear implant are not effective treatment. In fact, there is no well-established clinical treatment yet, and NIHL could not be completely prevented.⁷⁹ Therefore, the exploration of effective interventions to prevent or treat NIHL is urgent and critical.

Evidence-based treatment for SNHL in human

The administration of high dose Mg²⁺⁸⁰ and vitamin B12⁸¹ could prevent or reduce temporary hearing threshold shift caused by noise exposure. In a clinical study on NIHL, an anti-apoptotic cell-permeable JNK ligand, AM-111, was administered *via* intra-tympanic injections, demonstrating a marked therapeutic effect in some cases.⁸² The combination of steroid (prednisolone) and the nootropic drug (piracetam) also proved to be an effective treatment for gunshot NIHL.⁸³

Evidence-based treatment in animal

Pharmaceutical treatment

Retinoic acid, an anti-apoptotic agent and c-Jun N-terminal kinase (JNK)-inhibitor, was showed to have a protective effect against NIHL when locally injected into the inner ear of guinea pig and mice, possibly through blocking apoptotic cascades, such as the MAP kinase (MAPK)/JNK pathway.⁸⁴⁻⁸⁷ Glutamate antagonist and an NMDA receptor antagonist could also reduce the noise-induced damage on the inner ear of guinea pig and chinchillas.⁸⁸⁻⁹⁰

The blockade of calcium overload pathways could prevent the development of NIHL in guinea pig⁹¹ and mice.^{92, 93} Neurotrophins was also proved to be an effective treatment for NIHL in guinea pig.⁹⁴⁻⁹⁷

Antioxidants were demonstrated as able to reduce hearing loss in different animal models of NIHL if given before noise exposure, such as glutathione (GSH),^{98, 99} ebselen,¹⁰⁰ ascorbic acid,^{101, 102} and water-soluble coenzyme Q10¹⁰³ in guinea pig; resveratrol in rats;¹⁰⁴ D-methionine in a variety of other species;¹⁰⁵ but they were ineffective to reduce temporary threshold shift in human.¹⁰⁶

Molecular engineering

After inner ear damage, human hair cells and auditory neurons have little or no ability to regenerate, but progressively degenerate due to gradually decreased level of neurotrophins in the inner ear.¹⁰⁷ An ideal treatment would be the regeneration or replacement of the non-functioning hair cells and the auditory neurons. However, in current clinical practice, the pharmaceutical agents for NIHL are usually not cell-specific. The medications are given systemically or topically, but the drug concentration in the inner ear are not high enough to change gene expression and induce repair or regeneration. Molecular engineering might provide a solution.

Molecular engineering includes gene therapy and allotransplantation of stem cell. Gene therapy is to manipulate the gene expression of target cells, either by inducing the expression of a normal gene or by inhibiting the expression of a mutant gene, aiming to achieve phenotypic restoration of cell function.¹⁰⁸ In order to fully understand how gene therapy works *in vivo*, mammalian animal models are needed.¹⁰⁸

Gene therapy

In gene therapy, researchers need to identify the genes of interest, characterize the genes' roles in the regeneration of hair cells and auditory nerve; they need to select an appropriate vector, which can carry the genes into the cells. The most common vectors are viruses, plasmids that are capable of passing through cell membrane. Gene therapy has shown some promising results in animal models. In

1999, Math1 was found to be an essential gene in the development of the inner ear.¹⁰⁹ In a mouse model, the transfection of neurotrophin-3 gene showed that neurotrophin-3 could protect auditory nerves and hair cells from the ototoxic effect of cisplatin.^{110, 111}

A vector-based transgene expression of neurotrophic factor was found to prevent the degeneration of hair cells.¹¹² Furthermore, it was found that the transgene expression of neurotrophic factor (BDNF) or neurotrophin-3, which were mediated by exogenous vectors, could increase the survival time of auditory neuron.^{94, 97, 107} In a SNHL model, Shibata *et al*¹¹³ found that the expression of neurotrophin or BDNF gene in the resting epithelial or mesothelial cells would induce neural regeneration; the authors observed a robust regrowth of nerve fibers towards the neurotrophin-secreting cells, at last cell re-innervation would occur. Hence they concluded that neurotrophic factor or neurotrophin can protect hair cells, increase the survival time of auditory neuron and induce neural regeneration.

Recently, a new gene, Atoh1, has been found to be important for hearing function. A mouse model of congenital SNHL was established by Atoh1 gene knock-out. Atoh1 was then cloned into a vector, and delivered back into this mouse model. The transgene expression of Atoh1 was found to convert the supporting cells into ectopic sensory cells, which were similar to the endogenous sensory epithelia.^{114, 115}

A mouse model of SNHL was established by knocking out vesicular glutamate transporter-3 gene (VGLUT3). In the SNHL mouse model, there was almost no auditory-evoked brain stem response, the threshold of which was about 90 dB, and no startle response. A transgene expression was performed in which VGLUT3 gene was delivered back into the animal ear. Two weeks after transgene expression, the threshold of auditory-evoked brain stem response was normalized, and startle response was partially recovered. At last, morphologic study showed that the inner hair cell and ribbon synapse had been at least partially rescued. These findings represent a successful restoration of hearing function by gene therapy in a mouse model, which is a significant step toward gene therapy for human deafness.¹¹⁶

In an animal model, several options are available for delivering the genes of interest into the inner ear, for example, cochleostomy (open a hole on cochlear's lateral wall), canalostomy (open a hole on semicircular canals), or penetration of the round window's membrane.¹⁰⁸ However, for human therapy in the future, the best method should be minimally invasive.

Stem cell therapy

Another important method of molecular engineering is stem cell transplantation, which is to replace the missing target cells by allotransplantation of multipotent progenitors. It has been used in animal models to replace non-functioning auditory neurons.⁴ Neural stem cells, derived from the olfactory bulb of C57BL, were transplanted into the inner ear of mice via the cochlear lateral wall, and were found to differentiate into auditory neurons with high efficiency.¹¹⁷

In another study, Choi *et al*¹¹⁸ established a guinea pig model of SNHL by injecting ototoxic drug (neomycin) into the ear, an increased auditory-evoked brain stem response threshold was noticed (80–90 dB), the degeneration of outer hair cells and the missing of auditory neuron were also identified under optical microscope. The mesenchymal stem cells from umbilical cord blood were collected from pregnant women, and were transplanted into the deaf animals through the brachial vein. Five weeks after stem cell transplantation, a significant improvement of auditory-evoked brain stem response threshold was found, from 80–90 dB to 40 dB; increased expressions of auditory neurons and hair cells were also noticed. They concluded that intravenous transplantation of mesenchymal stem cells from umbilical cord blood could improve hearing thresholds, increase the expressions of outer hair cells and auditory neurons.

Cell line is another therapeutic option. Cell line has been reported to generate highly differentiated structures, such as cilia, which are long extensions from cochlear hair cell surface. This may be a promising technique which can be used to generate functioning hair cells. If proliferation and differentiation can be well controlled, ample hair cells can be generated which can be transferred to the cochlea.¹¹⁹

PROSPECT OF MOLECULAR ENGINEERING

Clinical trials and animal studies on NIHL are generally carried out under some experimental conditions, and a given treatment may work well to reduce the damage effect for one noise exposure paradigm and not for another. Systematic review and analysis of clinical trials and animal studies are needed.

Some results from animal studies cannot be proved in human, but translation of findings from animal studies into the clinic is not impossible, and can be sometimes very successful. Based on a laboratory result, a clinical trial about a new drug was designed and carried out, in which the incidence of gentamicin-induced hearing loss was reduced by 75%.^{120, 121} Therefore, once sufficient information is

available, it will be possible to plan clinical trial based on animal studies, and eventually providing promising direction for establishing an effective treatment for NIHL.

Both gene therapy and stem cell transplantation have been successful in restoring hearing function in animal models. It is reasonable to believe that molecular engineering might be a useful treatment for profound sensorineural hearing loss, particularly for neural hearing loss. However, this new therapy is still at its beginning stage, a lot of work needs to be completed before it can be applied in clinic.¹²²

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