REVIEW ARTICLE

Environmental and genetic factors in age-related hearing impairment

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ABSTRACT. Age-related hearing impairment (ARHI), or presbycusis, is a complex disease with multifactorial etiology. It is the most prevalent sensory impairment in the elderly, and may have detrimental effects on their quality of life and psychological well-being. The aim of this paper is to give an overview of the current data on ARHI, focusing mainly on environmental agents and genetic predisposition in animal models and in humans. With improvement of our understanding of ARHI, treatment other than with amplification will be hopefully possible in the long term.

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INTRODUCTION

Age-related hearing impairment (ARHI), or presbycusis, is the most prevalent sensory impairment in the elderly (1). Several studies demonstrate that it may have detrimental effects on their quality of life and psychological well-being. Social isolation, depression, anxiety and cognitive decline are frequently observed in affected persons (2, 3). ARHI has long been considered part of the natural process of aging, and thus an incurable and unpreventable disorder. Nowadays, there is mounting evidence that it is a complex disease with multifactorial etiology, which involves both environmental influences, which last throughout the lifespan, and genetic predisposition. Much research effort has been put into elucidation of the environmental factors and large amounts of data are available regarding the effects of noise exposure, ototoxic medication, exposure to chemicals, chronic medical conditions, malnutrition, tobacco smoking and alcohol abuse, although some of these factors still remain controversial (4). Conversely, little is known about the role played by genetic factors, although the number of genetic studies on presbycusis has been increasing at a surprising rate in the last few years. Several genes encoding proteins specific to the ear have been identified, and genotypic differences found in tissue samples from both humans and animals can be observed by means of powerful molecular techniques.

The aim of this paper is to give an overview of current data relating to ARHI, focusing mainly on environmental agents and genetic predisposition in animal models and in humans. Various techniques of prevention and future therapeutic strategies are also discussed.

ENVIRONMENTAL FACTORS

Several environmental agents and medical conditions may accelerate age-dependent hearing loss, although with different degrees of importance and experimental evidence.

Noise

Several mechanical and metabolic mechanisms are responsible for noise-induced hearing loss (NIHL). Structural damage ranges from disturbance of the delicate stereocilia to tearing of the organ of Corti and eventual permanent hair-cell loss, which first involves the outer hair cells (OHC) and than the inner ones (IHC). Unfortunately, this structural damage is difficult to distinguish from that caused by aging alone (5, 6). Thus, interactions between noise exposure and ARHI are difficult to evaluate and still little known in human subjects, because of lack of control over extrinsic variables and for ethical reasons. Nevertheless, an additive or interactive effect has been postulated for a long time. Accordingly, these issues have been examined in the Mongolian gerbil, a well-studied animal model of both ARHI and NIHL. In aged, noise-exposed gerbils, total hearing loss can be predicted by the addition of ARHI and NIHL in dB (7), similar to the international standard on hearing loss allocation, ISO-2000 (8).

Key words: Aging, hearing impairment, presbycusis.

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Metabolic damage may be caused by excessive release of glutamate, which is the excitatory neurotransmitter that acts at the synapses of the IHC with the eighth nerve fibers. During high-level noise exposure, a large amount of glutamate is released into the synapses by the IHC, thus overstimulating the glutamate receptors of the dendritic terminals of the afferent fibers of the auditory nerve. Glutamate is toxic for the cells and causes the condition of excitotoxicity, characterized by swelling and rupturing of dendrite terminals (9).

A second metabolic mechanism damaging the cochlea is the generation of reactive oxygen species (ROS) and toxic free radicals during and after noise exposure (10). A significant increase in superoxide (O_2^-) along marginal cells of the cochlear *stria vascularis* has been observed after exposure to high-intensity (120-125 dB SPL) rock 'n' roll music (11). Similarly, high levels of hydroxyl radicals and increased lipid peroxidation have been documented after excessive acoustic stimulation with various types of noise (12, 13). The cochlear hair cells are highly demanding of energy, particularly the OHC, because of their active motility. During noise exposure, the mitochondria use more and more oxygen to meet the increasing cellular demand for energy, thus generating more and more superoxide as an unwanted byproduct. The increased superoxide reacts with other molecules to generate higher levels of other ROS in the cochlea. ROS and free radicals can damage mitochondrial DNA (mtDNA), breaking down lipid and protein molecules and triggering cell death. Cell death may occur either as necrosis, or apoptosis, the existence of which in the noise-damaged cochlea was only described recently (14).

Lastly, another effect of noise trauma is reduced cochlear blood flow (CBF) as observed in many studies using a variety of noise exposure and blood flow detection techniques (15, 16). There is strong evidence that CBF reduction makes cells particularly vulnerable to several noxious agents, although whether this is a direct effect of noise or whether it is caused by increased levels of ROS induced by noise is still unclear.

Ototoxic medication

Aminoglycosides and cisplatin target the OHC in the basal turn of the cochlea, causing high-frequency sensorineural hearing loss in a substantial percentage of patients treated with these drugs. Each group of agents appears to generate ROS within the cochlea which trigger downstream mechanisms, leading to cell death (17). Another hypothesis of aminoglycoside ototoxicity implies a mechanism of action linked to excitotoxicity at the IHC/eighth nerve synapse, which is glutaminergic (18). Loop diuretics and high dosages of salycilates may cause hearing loss (17). Conversely, small doses of salycilates may improve hearing (19) and have a protective effect on aminoglycoside- and cisplatin-induced hearing loss. This effect is probably related to an hypothesized enhancement of OHC electromotility, or to an antioxidant effect of continuous low dosages of salicylates (20).

Chemicals

Workers exposed to solvents such as toluene, trichloroethylene, styrene and xylene reveal an increased prevalence of high-frequency hearing loss compared with non-exposed subjects (21). Moreover, even small doses of toluene can produce damage of the inner ear, and the associated hearing loss is only slightly lower than that produced by much higher doses of the solvent (22).

Alcohol abuse, tobacco smoking and diet

Heavy drinkers are at risk for a more severe form of presbycusis with respect to light drinkers (23), as moderate alcohol consumption is inversely correlated with hearing loss (24, 25). This effect seems to reproduce the well-documented association between drinking habits and cardiovascular diseases.

The effect of smoking is still controversial. In a recent study (26), smoking and cardiovascular disease (clinical and subclinical) as factors did not substantially influence the age-related hearing loss. Instead, Fransen et al. (25) observed that smoking did significantly increase high-frequency hearing loss, that the effect is dose-dependent and remained significant when cardiovascular disease events were taken into account. Similar results were reported by Cruickshanks et al. (27), who stressed the fact that changes in smoking habits can prevent or delay age-related decline in hearing sensitivity. A high body mass index has also been correlated with hearing loss across the frequency range tested (25). In conclusion, several studies demonstrate that a healthy lifestyle can protect against ARHI.

Head trauma

Head trauma is generally responsible for high-frequency hearing loss, which is slowly recovered over many months. Nevertheless, several studies have documented less frequent, but irreversible lesions, both in the peripheral and central auditory system. These lesions may contribute to accelerated AHRI (28).

Immune system

Iwai et al. (29, 30) carried out several experiments on an animal model for accelerated senescence, i.e. SAMP1 mice, which suffer from hearing impairment and decreased immune function, and demonstrated a significant association between immune system dysfunction and ARHI.

Diabetes

The association between diabetes and AHRI is still controversial, although some studies demonstrate that diabetes may act synergistically with several processes re-

sponsible for ARHI (31). There is stronger evidence that sensorineural hearing loss is more common in diabetic patients than in their age-matched controls, and the majority of differences between the two groups are found in measures of inner ear function (31-34). In a subgroup of these diabetic patients, AHRI is probably related to mtDNA mutations (35).

Cardiovascular disease

The inner ear is particularly vulnerable to hypoxia, as demonstrated in both animals and humans who suffered from sudden deafness. Chronic cochlear hypoxia may cause an accumulation of mtDNA mutations which significantly reduce energy processes and in turn decrease the endocochlear electric potential, i.e., the so-called strial or metabolic presbycusis. Acute ischemic episodes are a frequent cause of irreversible damage of both central and peripheral auditory systems, which contributes to accelerated presbycusis. In a recent study Nomiya et al. (36) observed that, in temporal bones from subjects with generalized arteriosclerosis, the mean loss of OHC and of spiral ganglion cells was significantly greater than that of normal controls, and that the *stria vascularis* and spiral ligament were severely atrophic. This cochlear degeneration, especially in the basal turn, was already apparent in young adults with generalized arteriosclerosis.

GENETIC FACTORS

The hypothesis that genetic factors may play a significant role in AHRI was proposed as early as the 1970s by Paparella et al. (37), although as an anecdotal observation. At that time, no experimental evidence was feasible and the hypothesis was mainly based on two facts: the observation of several families whose members were affected by presbycusis, which manifested very early, occasionally before the age of 45, and the consideration that both the time of onset and the rate of progression of presbycusis vary greatly between individuals. The ISO database for the hearing threshold (8) quotes a hearing loss at 4 Kz, showing a variation of 50 dB (10-90 percentiles) for 70-year-old male subjects. This marked variance is difficult to explain only by the effects of environmental influences or chronic medical conditions, and is thus a further support to the existence of a genetic predisposition. Another demonstration of the role of genetic background is the existence of various types of phenotypic presbycusis. A classification of the diverse forms of presbycusis derives from more than 50 years of study by Schuknecht, who correlated histopathological observations from post-mortem temporal bone specimen and audiometric data obtained in previously living old patients (38, 39). This classification has been criticized because of its limited usefulness in clinical practice. However, the distinction of: a) sensorial, b) neural, c) metabolic, d) mechanical and e) mixed presbycusis still maintains its speculative and theoretical interest (40, 41). The first type, sensory presbycusis, is classically considered to be characterized by ciliate cell degeneration in the basal end of the cochlea and associated with high-frequency hearing loss. New findings regarding the kinds of cells involved in this form of presbycusis have included more non-sensory and supporting cells. Nelson and Hinojosa (42) observed that individuals with this audiometric pattern of presbycusis exhibit degeneration of the *stria vascularis*, spiral ganglion cells, IHC and OHC all associated with the severity of hearing loss. The second type, neural presbycusis, involves a loss of cochlear neurons and poor word discrimination in the presence of stable pure-tone thresholds. The third type, metabolic presbycusis, is associated with atrophy of the *stria vascularis* and hearing loss, with a flat pure-tone audiometric threshold and good word discrimination scores (43). The fourth type, mechanical presbycusis, is characterized by normal morphologic findings and a linear descending pure-tone audiogram, which is considered to be related to abnormal motion mechanics of the cochlear partition such as stiffening of the basilar membrane. The fifth type, mixed presbycusis, shows combinations of flat, gradually sloping, and abrupt high-tone hearing loss with observable light microscopy abnormalities of multiple cochlear elements.

In the 1990s, the relative contribution of genetic factors with respect to environmental influences was clearly demonstrated in humans by three studies on ARHI heritability. Measures of heritability reflect the percentage of the phenotypic variance which is due to genetic factors. Karlsson et al. (44) evaluated 557 male twins, demonstrating that 47% of the variance of the presbycusis phenotype after age 64 was attributable to the effects of genes. In 1999, Gates et al. (45), in a study with members of the Framingham cohorts, suggested a heritability ranging from 35 to 55% for sensory presbycusis and from 25 to 42% for strial presbycusis. Lastly, a Danish study identified a heritability of 40% for self-reported hearing loss in twins over 75 (46). As reported by the authors, these analyses cannot differentiate the contributions of genes alone from those which may involve gene-environment interactions, but the heritability estimates were strong.

An important contribution to the study of genetic factors in ARHI derives from laboratory researches on genetically modified animals. The fact that some mouse strains are very susceptible to ARHI was established over 20 years ago. Since then, mice have become the most widely used animals because of their high vulnerability to age in several inbred strains, and minimal variance within any strain. Characterization of these ARHI strains demonstrates that both progressive high-frequency hearing loss and age-dependent inner ear degeneration are very similar to that shown in human presbycusis. The most prominent morphological features include degen-

eration of the organ of Corti and variable loss of cells in the spiral ganglion and the cochlear lateral wall. While most of these strains most closely resemble sensory ARHL, recent work has identified mice possessing the essential characteristics of neural and strial ARHL, thus further supporting Schuknecht's framework (40).

Johnson et al. (47) demonstrated that early presbycusis in one of these strains, i.e., C57BL/6J (abbreviated B6), is associated with a single major recessive gene located on chromosome 10. This gene was named age-related hearing loss or *Ahl*. In the following years, two other loci, *Ahl2* and *Ahl3*, were identified (48, 49), and very recently *Ahl4* has been located on the distal chromosome 10 in A/J mice (50).

Several studies demonstrate that B6 mice are homozygous for the defective Cdh23 ahl allele of the gene encoding cadherin 23 (51-53). This allele, a hypomorphic single nucleotide polymorphism (SNP), is also common to nine other inbred mouse strains in a recessive way (54). Cadherins are calcium-dependent proteins which glue together cells of the same type in order to form tissues. In the cochlea, otocadherins, or CDH23, are located in the stereocilia of the OHC and hold the cilia together. The lateral links between the sterocilia (55) are probably composed of otocadherins (56). The lack or absence of otocadherins increases the vulnerability of hair cells to both aging and noise exposure. The function of otocadherins is also closely correlated with calcium concentrations around the stereocilia (typically 7 calcium ions per molecule of cadherin). Small fluctuations in this concentration affects the ability of the otocadherins to form the lateral links between the stereocilia (56). At the same time, calcium homeostasis is closely regulated by the plasma membrane Ca^{2+}-adenosine triphosphatase type 2 pump (Atp2b2, also known as Pmca2). This pump removes calcium outside the cell through the cell membrane, and its role is particularly important during noise over-exposure. In fact, high levels of noise significantly increase calcium concentrations inside the stereocilia and hair cells (57), reaching levels which may then be toxic to cells.

The gene encoding this pump protein is mutated in the deafwaddler mouse mutant (58, 59), which is deaf, waddles when walking, and bobs its head. The phenotype of this strain of mice is also influenced by a modifier gene (51). Many other modifier genes are also probably implicated in regulations of inner ear functions, thus demonstrating the complexity of AHRI etiopathogenesis.

Experimental data obtained in mice have been compared in humans. "Cross-talk" between studies in humans and those in model organisms is essential in this field. Unfortunately, of the many candidate genes presumed to increase vulnerability to AHRI or AHRI and noise in mice, only a few have been confirmed to play a role in humans.

So far, two genome-wide-linkage studies have been performed, resulting in the location of seven different susceptibility regions for ARHI (60, 61). A number of association studies on candidate susceptibility genes have also been performed. A strong association was detected between several SNPs of the gene KCNQ4 and ARHI in two independent European subject groups (62). KCNQ4 is a gene, located on chromosome 1, encoding a protein for a potassium channel which is thought to play a critical role in regulating neuronal excitability, particularly in sensory cells of the cochlea. Missense mutations of this gene, described in several families, are a cause of non-syndromic sensorineural autosomic dominant deafness type 2 (DFNA2) (63), the phenotype of which is characterized by high-frequency hearing loss, with an average progression of 1 dB/year.

An association tested by various authors regards ARHI and the genes that contribute to defence against oxidative stress, which is caused by an imbalance between the production and removal of ROS. The exact mechanism by which ROS may cause ARHI remains unknown. There is evidence that ROS levels in the inner ear increase after exposure to noise, ototoxic compounds and ear diseases, and as an effect of age-dependent degeneration. The combined effects of these insults during the lifetime lead to hair-cell damage. Antioxidant enzymes, such as glutathione and related molecules (GST and GSH) are present in the inner ear (64) and play a central role in the metabolism and inactivation of ototoxic agents (65). Sha et al. (66) demonstrated that the basal cochlear partition is more vulnerable to damage by free radicals because GSH levels in the OHC are lower than in intermediate and apical cochlear portions.

Unal et al. (67) and Van Eyken et al. (68) succeeded in finding a strong association with a polymorphism in N-acetyltransferase 2 (NAT2_6A), which is an important enzyme protecting the inner ear against oxidative stress. An association of GSTM1 (glutathione s-transferase, mu-1) and GSTT1 (glutathione s-transferase, theta-1) deletion polymorphisms and ARHI in the Finnish population has also been demonstrated (68). Lastly, the apparent protective effect of apolipoprotein E (APOE) allele 14 has recently been suggested (69).

High levels of oxidative stress appear to be highly correlated with increased mtDNA mutations in the inner ear. These mutations have long been known to be associated with human diseases, including non-syndromic hearing loss (70), and their significant increase has been described in aging human auditory tissue (71). Analyses of human temporal bones have shown that a 4977 bp deletion, the so-called "common deletion" (CD), is a frequently acquired mitochondrial mutation in ears affected by ARHI, but is not observed in age-matched control subjects not affected by presbycusis (72-74). These data were confirmed by a similar mutation in

AHRI rats (76). A clear relationship also exists between quantitatively measured levels of the CD and the severity of hearing loss (73). The same authors (75) have recently observed that, in addition to the CD, other deletions in spiral ganglion cells may trigger cell apoptosis and be even more important than CD level alone with respect to deficits in cellular energy metabolism, thus playing a significant role in pathogenesis of presbycusis. Several deletions involving the mtDNA major arc contribute to the observed deficit in expression of cytochrome c oxidase subunit 3 (COX 3). The COX complex is located in the inner mitochondrial membrane and is the last component of the respiratory electron transport chain, which generates the transmembrane electrochemical gradient essential for the production of ATP. A deficiency in COX activity leads to significant pathology in highly metabolic tissues.

Another aspect which has long been investigated, but with controversial results, is the relationship between the melanin-producing intermediate cells of the cochlear *stria vascularis* and individual susceptibility to AHRI. These cells play a critical role in producing the endocochlear potential (EP) and in maintaining the high levels of K^+ that normally exist in *scala media*. A recent study by Ohlemiller et al. (76) demonstrates that a marginal cell loss or dysfunction may cause strial presbycusis, probably through progressive decline of the EP. The authors conclude that, in any individual, the combined effects of genetic background, environmental influences and stochastic events determine whether marginal cell density or function falls below some critical level, thus producing a significant decline of the EP.

Other authors have observed that a normal concentration of eumelanin (the major form of melanin) may have a protective effect against age and noise damage, *via* antioxidant and metal chelating effects (77). The melanin produced by intermediate cells is exported to the intrastrial space, where it can be taken up by strial marginal and basal cells. Instead, pheomelanin, a melanin isoform, seems to aggravate oxidative processes (78).

Genome-wide association study (GWAS) refers to the analysis of hundreds of thousands of genetic markers, either in a single individual, or in pools of thousands of well-characterized case and control subjects. The latter method has clarified the genetic underpinning of several diseases such as breast cancer, Crohn's disease and type 2 diabetes. Unfortunately, whole genome analysis of ARHI has been hampered in the past by the limitations of genotyping technology and its prohibitive cost. Although, the development of more powerful microarrays, new methods of statistical analysis, and SNP genotyping have partially reduced the costs in the last few years, a candidate gene approach is still used in AHRI research to identify susceptibility genes.

Genes causing monogenic forms of hearing loss are excellent susceptibility candidates for ARHI. For the moment, two different GWAS for AHRI have been performed in Europe with a vast pool of subjects. The first (79) resulted in the identification of a highly significant and replicated SNP, possibly associated with ARHI, located in GRM7, the gene encoding metabotropic glutamate receptor type 7 (mGluRT7). This gene is expressed in the hair and spiral ganglion cells of the inner ear, and its variation is probably related to an individual's risk of developing AHRI through a mechanism of altered susceptibility to glutamate excitotoxicity. L-glutamate, the primary excitory amino acid neurotransmitter in the mammalian central nervous system, is expressed in the synapses between the IHC and auditory afferent neurons. High concentrations of glutamate are neurotoxic because of its excitatory properties, and this effect has been implicated in several forms of progressive hearing loss, including NIHL and AHRI. Because mGluRT7 reduces release of glutamate, the authors hypothesize that the causative allele of GRM7 may alter synaptic autoregulation of glutamate in the synaptic cleft between IHC and auditory neurons. Over time, these higher levels of glutamate may lead to excitotoxic neuronal and/or sensory cell death.

Lastly, the second study identified a highly significant SNP in the grainyhead-like 2 gene (GRHL2) (80). A mutation in GHRL2 is known to be responsible for DFNA28 autosomal dominant hearing loss (81). The hearing loss in the family segregating this mutation may be categorized as mild to moderate across all frequencies in the initial stages, but progresses toward severe hearing loss of high frequencies in the fifth decade. The age at onset is variable; the youngest patient was diagnosed in his first decade. As such, DFNA28 hearing loss does not completely match the typical features observed in ARHI, but some properties certainly correspond, such as the progressive, sensorineural nature, and the fact that high frequencies are most affected later in life.

PREVENTION AND TREATMENT

The identification and isolation of AHRI genetic factors may have practical outcomes. For example, subjects genetically predisposed should be more closely protected against noise, trauma, aminoglycosides, solvents and other toxic agents, compared with recommendations for non-predisposed individuals.

There is also mounting evidence that the ear is particularly vulnerable to noise during its maturational stage and that exposure to noise during childhood may be responsible for increased vulnerability to aging (82). Thus, policies and procedures to minimize excessive noise exposure since the early months of life should be adopted, and education about noise and its effects on hearing, health and learning should begin in elementary school (83).

Until recently, the cochlea has been considered to lack the capacity to regenerate new sensory and neural cells (39), but recent experimental investigations indicate that hair cell regeneration in the inner ear is possible (84). Research into the mechanism of age-related hearing loss has led to a concomitant search for substances to protect or rescue hair cells from noxious agents, such as free radical scavengers, glutamate receptor antagonists, anti-apoptotic agents and neurotrophic factors. These agents have been used with mixed results in mouse and rat models (85). These animals have been raised on a calorie-restricted diet, with successful results. In addition, in the Mongolian gerbil model, lost endocochlear potential voltage has been restored by exogenous electrical stimulation. In humans, the only successful double-blind, placebo-controlled clinical trial, with a protective agent to prevent ototoxicity, was carried out in China (19). Acetylsalicylic acid or placebo was given in combination with gentamycin and a significant decrease in hearing loss was observed. Unfortunately, clinical trials in humans are still hampered by many technical difficulties. In order to provide the maximal dose of protective agent without systemic interference with the desired effect of the anti-infective agent or anti-neoplastic drug, these protective agents need novel methods of administration, such as injection within the middle ear. A few initial results are encouraging, such as the development of cochlear implants combined with local drug/gene devices or stem cell therapy. These new devices may improve auditory performance by inducing cellular/neuronal protection and/or regeneration/resprouting and by promoting cochlear regeneration (86).

CONCLUSIONS

Presbycusis is a complex disease with multifactorial etiology, which involves both environmental influences and genetic predisposition. Genome-wide studies on AHRI are now feasible and although still expensive, the costs are no longer prohibitive, because of recent progress in microarray technology, new methods for SPN genotyping and improved statistical analysis. Genome-wide linkage studies have resulted in seven candidate susceptibility regions for AHRI (60, 61) and many more are expected in the next few years, while association studies have revealed the first two genes involved in AHRI (67, 68). Identification of these genetic factors will permit a more personalized prevention against various exogenous noxae in genetically predisposed subjects. Hair cell regeneration in the inner ear does seem to be possible, and substances to protect or rescue hair cells have been used with mixed results in animal models. In humans, clinical trials are still hampered for technical and ethical reasons, but a few initial results are encouraging. Thus, treatment other than with amplification will hopefully be possible in the long term.

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