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The Complexity of Age-Related Hearing Impairment: Contributing Environmental and Genetic Factors

E. Van Eyken G. Van Camp L. Van Laer

Department of Medical Genetics, University of Antwerp, Antwerp, Belgium

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Ageing · Hearing impairment · Presbycusis

Abstract
Age-related hearing impairment (ARHI) is the most common sensory impairment seen in the elderly. It is a complex disorder, with both environmental as well as genetic factors contributing to the impairment. The involvement of several environmental factors has been partially elucidated. A first step towards the identification of the genetic factors has been made, which will result in the identification of susceptibility genes, and will provide possible targets for the future treatment and/or prevention of ARHI. This paper aims to give a broad overview of the scientific findings related to ARHI, focusing mainly on environmental and genetic data in humans and in animal models. In addition, methods for the identification of contributing genetic factors as well as possible future therapeutic strategies are discussed.

Introduction

Age-related hearing impairment (ARHI), also referred to as presbycusis, is the most common sensory impairment seen in the elderly. The overall population in developed countries is ageing; therefore, an increasing proportion will develop ARHI in the nearby future. People affected with ARHI often experience difficulties adjusting to their disorder. In fact, hearing loss may have a major impact on the quality of life and the psychological well-being of affected persons. Communication difficulties will lead to poor psychosocial functioning, leading to social isolation as a consequence. The affected person may lose his or her independence, and suffer from depression, anxiety, lethargy, social dissatisfaction and possibly a cognitive decline, similar to dementia, as a consequence [Dalton et al., 2003; Gates and Mills, 2005; Heine and Browning, 2002].

It has become clear that ageing causes histological, electrophysiological and molecular changes. Deficits in hair cells, cochlear neurons and the stria vascularis, and combinations thereof have been described. In its most typical presentation, ARHI is symmetrical, sensorineural and more pronounced in the high frequencies. ARHI has been classified into four types, referred to as Schuknecht's topology [Schuknecht and Gacek, 1993], including sensory ARHI (high-frequency loss; loss of sensory cells), strial or metabolic ARHI (flat descending threshold pattern; atrophy of stria vascularis), neural ARHI (loss of word discrimination; loss of cochlear neurons) and cochlear conductive or mechanical ARHI (unknown pathology). In reality, many ARHI subjects show mixtures of these pathologies, which is referred to as mixed ARHI. When multiple influences interact, their effects are considered to be additive [Schuknecht and Gacek, 1993]. Although Schuknecht's classification is a

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G. Van Camp
Department of Medical Genetics, University of Antwerp, Campus Drie Eiken
Universiteitsplein 1
BE–2610 Wilrijk (Belgium)
Tel. +32 3 820 24 91, Fax +32 3 820 25 66, E-Mail guy.vancamp@ua.ac.be

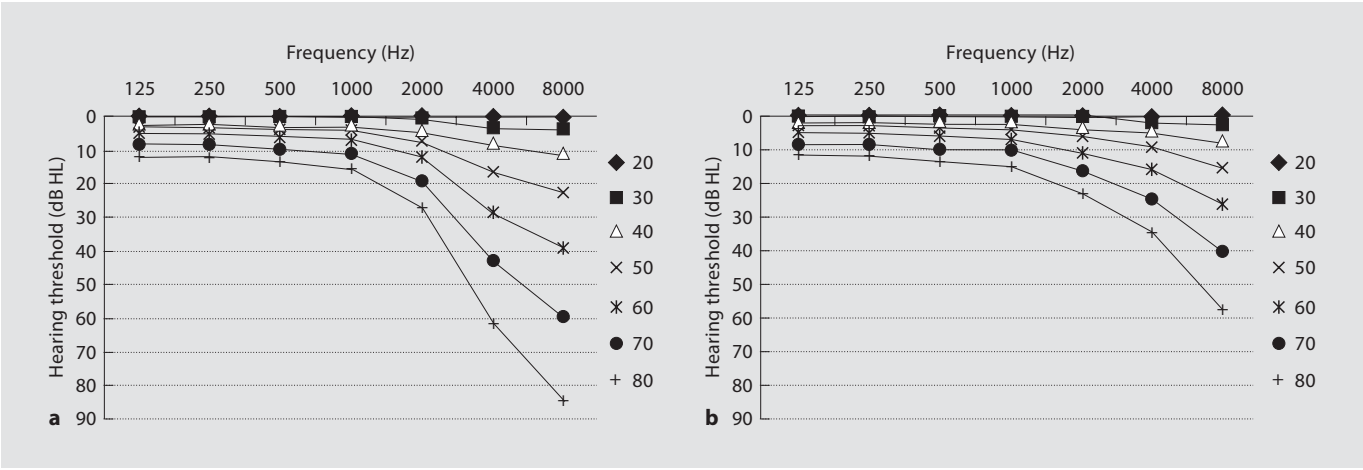

Fig. 1. ARHI according to the ISO 7029 standard for males (**a**) and for females (**b**). The x-axis displays the frequencies (Hz) and the y-axis displays the hearing thresholds (dB HL). Each particular graph is representative of the median of the hearing thresholds at a given frequency for a particular age (ranging from 20 to 80 years old with an increment of 10 years).

Table 1. Prevalence of ARHI

ARHI prevalence	References
At the age of 61–70, 37% have a significant HI of at least 25 dB At the age of 71–80, 60% have a significant HI of at least 25 dB	Davis [1989, 1994]
90% of all females with HI are more than 60 years old 50% of all males with HI are more than 60 years old	Davis [1991]
Males are more affected than females	Cruickshanks et al. [1998b]; Davis [1994]; Helzner et al. [2005]; Pearson et al. [1995]
At the average age of 65, 50% have HI	Cruickshanks et al. [1998b, 2003]
65-year-olds account for 37% of all hearing loss	Desai et al. [2001]
60% of all 73- to 84-year-olds have HI High frequencies are mainly affected	Helzner et al. [2005]

HI = Hearing impairment.

useful guideline, which incorporates testable hypotheses, refinement of these classifications is occasionally necessary due to the availability of increased knowledge regarding genetic defects and environmental conditions [Ohlemiller, 2004].

To date, several studies to estimate the prevalence of ARHI have been performed. An overview is given in table 1. In 1999, the World Health Organization estimated that worldwide 580 million people over the age of 60 suffered from hearing loss. Due to the growing population of elderly, it is expected that in 2020 this number will have increased by 75%, meaning that over 1 billion people of 60 years or older will be affected with ARHI (http://www.who.int/en/).

The prevalence of ARHI grows with increasing age. Overall, hearing thresholds aggravate on average with 1 dB per year for persons over 60, depending on age, gender and initial thresholds [Lee et al., 2005]. The largest variation of the hearing loss is found at the high frequencies, and at the older ages (fig. 1) [International Organiza-

tion of Standardization, 2000]. In general, males are more severely affected than females. The median hearing threshold shifts relatively compared to a group of persons aged 18 years (= the audiometric zero) have been described in the International Standard ISO 7029 [International Organization of Standardization, 2000]. In addition, ISO 7029 gives the expected statistical distribution above and below the mean values for a group of normal-hearing subjects within the age range of 18–70 years at each audiometric frequency ranging between 125 to 8000 Hz. As such, ISO 7029 is a very useful resource for the evaluation of audiograms in function of age and sex, if the screening method employed for the populations that were used to compile this standard is taken into account.

Hearing aids are the only therapeutic treatment available for ARHI at the moment, and are only helpful for a restricted group of affected people. They are capable of amplifying sounds, but speech recognition gain is often experienced as poor, particularly in noisy environments. Hearing aids are not used by all who would benefit from them. This is partly due to social attitudes that undervalue hearing, the cost, and the fact that many people refuse to use a hearing aid due to social stigmatization [Gates and Mills 2005]. Therefore, the development of new therapeutic strategies is necessary.

With this review, we will mainly focus on ARHI as a complex disease influenced by genetic as well as environmental factors. We will also briefly describe some ARHI mouse models and therapeutic possibilities.

ARHI Is a Complex Genetic Disorder

Even though every individual shows a steady decline in hearing ability with ageing, there is a large variation in age of onset, severity of hearing loss and progression of disease, which results in a wide spectrum of pure-tone threshold patterns and word discrimination scores. ARHI has always been considered to be an incurable and an unpreventable disorder, thought to be part of the natural process of ageing. Nowadays, ARHI is recognized as a complex disorder, with both environmental and genetic factors contributing to the etiology of the disease. This also means that it is not an inevitable disorder. Instead, ARHI should be considered as any other complex disease with a possible treatable and/or preventable nature. Scientific research should aim at the elucidation of the contributing factors. Many studies on environmental risk factors have been performed, which is in contrast with the limited number of studies that have attempted to identify genetic susceptibility factors contributing to the disease.

The heritability of a disease expresses the relative importance of the genetic component of a disease. It is defined as the proportion of the phenotypic variance attributed to the effect of genes. Karlsson et al. [1997] performed a twin study that estimated heritability values for ARHI. They studied 250 monozygotic and 307 dizygotic twins (aged between 36 and 80), using a questionnaire and audiometric data. The heritability for the age group above 64 was 0.47, indicating that about half of the population variance for this age category is due to genetic factors, while the other half is due to environmental factors. Another study compared audiometric data from genetically related subjects with those from genetically unrelated subjects. This study also revealed a familial aggregation for ARHI and resulted in heritability estimates between 0.35 and 0.55, depending on the frequencies that were analyzed [Gates et al., 1999]. Finally, a Danish twin study tested the heritability of self-reported hearing loss in mono- and dizygotic twins of 75 and older. In the latter study, a heritability of 40% was estimated [Christensen et al., 2001].

It is not known how many environmental and genetic factors contribute to the etiology of the disease, how they interact with each other and what their individual contribution is. The next section will aim to give an overview of the environmental and genetic factors that are known today, and the approaches that are used to identify genetic factors.

Environmental and Medical Factors

Environmental Factors

Extensive research has been carried out so far to elucidate the contribution of several environmental risk factors to ARHI, such as noise exposure, medical conditions, exposure to chemicals, ototoxic medication, hormones, alcohol and tobacco intake (table 2).

Noise Exposure

The most extensively studied environmental factor is noise exposure, which is responsible for both mechanical and metabolic damage to the cochlea [Flock et al., 1999; Mulroy et al., 1998; Pujol and Puel, 1999; Yamasoba et al., 1998]. A daily exposure to noise of 85 dB or higher elevates the risk of noise-induced hearing loss, leading to a primary loss of outer hair cells followed by inner hair cell

Table 2. Environmental factors

Environmental factor	Comments on risk factor	References
Noise	Leisure noise causes ARHI	Clark [1991]; Lutman and Spencer [1990]
	Gunfire noise causes ARHI	
	Noise exposure increases vulnerability to ARHI	Gates et al. [2000]; Kujawa and Liberman [2006]
Chemical exposure	Toluene, trichloroethylene, styrene, xylene cause ARHI	Johnson and Nylen [1995]; Fuente and McPherson [2006]; Fuente et al. [2006]; Morata et al. [2002]
	Causes ARHI in combination with noise exposure	Rybak [1992]; Chang et al. [2006]; Fuente and McPherson [2006]; Fuente et al. [2006]; Sliwinska-Kowalska et al. [2004]
Tobacco	Tobacco use: increased risk	Mellstrom et al. [1982]; Rosenhall et al. [1993]; Helzner et al. [2005]; Cruickshanks et al. [1998a]; Itoh et al. [2001]; Nomura et al. [2005]
	Tobacco use: no effect	Brant et al. [1996]; Gates et al. [1993]; Fuortes et al. [1995]
Alcohol	Alcohol abuse: increased risk	Rosenhall et al. [1993]; Helzner et al. [2005]
	Alcohol abuse: no effect	Brant et al. [1996]; Itoh et al. [2001]
Ototoxic medication	Aminoglycosides, cisplatin, salicylate, and loop diuretics cause ARHI	Stypulkowski [1990]; Aran et al. [1992]; Boettcher et al. [1992]; Mills et al. [1999]; Chen et al. [2006]; Lee et al. [1998]; Rybak et al. [2007]; Selimoglu [2007]
Medical conditions	Renal failure	Antonelli et al. [1990]
	Diabetes	Kurien et al. [1989]; Frisina et al. [2006]
	Cardiovascular disease	Kurien et al. [1989]; Gates et al. [1993]; Brant et al. [1996]; Picciotti et al. [2004]; Torre et al. [2005]
	High bone mineral density: protective effect	Clark et al. [1995]; Helzner et al. [2005]
	Head trauma causes ARHI	Danielidis et al. [2007]; Feldmann [1987]; Fitzgerald [1996]; Rosenhall et al. [1993]
	Immune function impairment is a risk factor	Iwai et al. [2003]; Iwai et al. [2001]; Iwai et al. [1999]
Diet	Nutritional intake	Houston et al. [1999]
	Caloric restriction: protective effect	Seidman [2000]
	Caloric restriction: no effect	Willot et al. [1995]; Torre et al. [2004]
	Antioxidant intake: protective effect	Le and Keithley [2007]
Hormonal factors	Estrogen and aldosterone have a protective effect; progestin causes ARHI	Guimaraes et al. [2004, 2006]; Hultcrantz et al. [2006]; Tadros et al. [2005]
Socioeconomic status	Lower social class, no higher education is a risk factor for ARHI	Sixt and Rosenhall [1997]; Poortinga [2007]

degenerations [Emmerich et al., 2000]. The contribution of noise exposure to the development of ARHI has initially been suggested by two studies performed in isolated African tribes living in relatively noise-free environments. ARHI appeared to be absent in those tribes [Jarvis and Van Heerden, 1967; Rosen et al., 1962]. This notion is not uncontroversial, since subsequent studies have shown that ARHI may be present in these cultures, albeit to a lesser extent [Driscoll and Royster, 1984]. In persons subjected to lifelong noise exposure, the audiological and histological differences between noise-induced hearing loss and ARHI are difficult to distinguish [Corso, 1992; Li, 1992]. Gates et al. [2000] suggested that noise exposure reduces the effect of ageing at the exposed frequencies, but that it accelerates the effect of ageing on hearing thresholds in adjacent frequencies. Indeed, it has been noted that the rate of ARHI in noise-damaged ears differs from the rate in nonexposed ears [Gates et al., 2000]. It is, therefore, thought that a predisposition for ARHI might be expressed at an earlier age due to noise exposure [Erway et al., 1996]. Kujawa and Liberman [2006] detected an increased vulnerability to ageing in inner ears of mice that were exposed to noise at a younger age. In addition, BALB/C and C57Bl/6J mouse models,

which show pronounced age-related hearing loss, are also more vulnerable to noise compared to CBA mice strains. Hence, genes associated with ARHI might contribute to the vulnerability to noise insults [Ohlemiller et al., 2000], whereas noise and age might have an additive or an interactive effect on hearing [Corso, 1992].

Exposure to Chemicals

Exposure to industrial chemicals such as toluene, trichloroethylene, styrene and xylene is considered a causative environmental factor for ARHI [Johnson and Nylen, 1995]. In addition, exposure to these chemicals often displays a nonlinear effect in combination with noise exposure (table 2) [Chang et al., 2006; Fuente and McPherson, 2006; Morata et al., 2002; Rybak, 1992; Sliwinska-Kowalska et al., 2004]. Therefore, the odds ratio to develop hearing loss not only increases with age [Morata et al., 2002; Sliwinska-Kowalska et al., 2004] but also with a lifetime exposure to solvents [Sliwinska-Kowalska et al., 2004].

Sliwinska-Kowalska et al. [2004] detected increased odds ratios for hearing loss in noise- and solvent-coexposed workers, exposed to a mixture of organic solvents, mainly existing of xylene isomers. Morata et al. [2002] described a higher prevalence of high-frequency hearing loss in workers exposed to styrene and noise and workers exposed to styrene alone, compared to noise-exposed and nonexposed workers. Styrene-exposed groups showed poorer hearing thresholds, even at small doses [Morata et al., 2002]. When compared to noise alone, elevated hearing thresholds were detected for workers exposed to toluene and noise [Chang et al., 2006]. Mainly the speech frequencies were affected, although the poorest hearing thresholds were measured at 6 kHz [Chang et al., 2006]. Similarly to styrene exposure [Morata et al., 2002], the hearing loss due to small doses of toluene was only slightly lower than that of highly exposed workers, indicating that even at low doses chemical solvents might damage the inner ear [Chang et al., 2006].

Smoking and Alcohol (Ab)Use

Smoking habits and alcohol (ab)use may also have an effect on the development of ARHI, although the study results are controversial (table 2). Cigarette smoking may increase the need for oxygen. As such, it may reduce the oxygen supply to the inner ear due to the presence of carbon monoxide. Cigarette smoke may also lead to a hypercoagulable state and coronary vasoconstriction, while the nicotine may induce hemodynamic effects [Ludvig et al., 2005]. Due to the reduced oxygen supply in the inner ear, the hypoxia may cause cochlear damage as well as an increased number of mitochondrial mutations, resulting in hearing loss.

A study performed by Itoh et al. [2001] found an association between drinking habits and hearing loss. Light, occasional drinkers appear to be protected against hearing loss, while heavy drinkers had an increased risk for developing ARHI. A similar association was also found for cardiovascular diseases (CVDs) and drinking habits, with a general health advantage for light drinkers and an increased risk for heavy drinkers [Wannamethee and Shaper, 1998].

Ototoxic Medication

Ototoxic medication frequently results in hearing loss in older subjects. This is probably due to an elevated utilization of medication and an altered renal and liver function in the elderly, with higher medication blood levels as a consequence. Examples of frequently used ototoxic medication are aminoglycoside antibiotics, chemotherapeutics like cisplatin, which cause nonreversible hearing loss [Aran et al., 1992; Boettcher et al., 1992], and salicylate and loop diuretics, which cause reversible hearing loss [Aran et al., 1992; Boettcher et al., 1992; Stypulkowski, 1990]. Possibly, the ototoxic effect of aminoglycosides and chemotherapeutics is induced by the formation of free radicals which could subsequently cause permanent damage to sensory cells and neurons [Chen et al., 2007; Rybak et al., 2007; Selimoglu, 2007]. This has been suggested because an antioxidant treatment preserves hearing in gentamicin-treated [Schacht, 1998], cisplatin-treated [Rybak et al., 2007] and aminoglycoside-treated subjects [Chen et al., 2007; Selimoglu, 2007]. Although the ototoxic effect of salicylate has been well documented, long-term administration of small doses of the drug may improve hearing [Huang et al., 2005]. The mechanisms behind this phenomenon are still unclear. It may be that long-term salicylate administration enhances cochlear mechanisms due to prestin upregulation or to an increased affinity of prestin for anionic ions which in turn may increase the motility of the hair cells. Alternatively, this could result from an increase in stereocilia transduction or in an altered cyclooxygenase activity [Huang et al., 2005]. In addition, salicylate as an antioxidant has a protective effect on aminoglycoside-induced hearing loss [Chen et al., 2007]. Some studies have demonstrated a gender-specific effect of certain drugs on the hearing levels in aged subjects [Lee et al., 1998; Mills et al., 1999], with females being more sensitive to treatment with certain drugs than males. Although the authors did not pro-

vide a good explanation for the differences between males and females, most probably factors like noise history or hormonal influences may be responsible. Because 80% of the males reported a history of noise exposure (females only 20%) [Mills et al., 1999], this may lead to damage to the male inner ear, which in turn may reduce the effect of the drug.

Medical Factors

Diabetes

Mitochondrial DNA mutations leading to a combination of late-onset diabetes and sensorineural hearing loss have been described [Janssen et al., 1999; Kurien et al., 1989]. Kakarlapudi et al. [2003] also noted that sensorineural hearing loss was more common in diabetic patients than in their age-matched controls. Moreover, young diabetic subjects (up to 60 years) have significantly more high-frequency hearing loss than healthy age-matched controls, but the difference in hearing loss between the two groups diminishes from 60 years of age onwards [Vaughan et al., 2006]. In addition, diabetes has been associated with ARHI [Kurien et al., 1989]. Presumably, diabetes acts synergistically with the processes involved in the development of ARHI [Kakarlapudi et al., 2003]. In a study investigating hearing loss in aged type II diabetics and age-matched controls, a significant difference between both study groups in inner ear and central hearing loss was found. Interestingly, in this study the lower frequencies tended to be more affected than the high frequencies [Frisina et al., 2006], while diabetes associated with hearing loss, as well as ARHI, is usually thought to lead to progressive high-frequency losses.

Cardiovascular Disease

Due to the high prevalence of cardiovascular disease (CVD) in ageing subjects, many studies have looked for a possible association between CVD and ARHI. In the Framingham cohort, for example, an association between low-frequency hearing loss and cardiovascular events was observed [Gates et al., 1993]. In addition, high-density lipoprotein levels were correlated with hearing thresholds, especially in women [Gates et al., 1993; Lee et al., 1998]. Torre et al. [2005] detected a gender-specific association between CVD and hearing loss in the elderly as well; women with a self-reported history of myocardial infarction were twice as likely to develop ARHI as women without a history of myocardial infarction. This was not observed in men. Again, these differences between genders may be due to hormonal differences. Hypertension and systolic blood pressure have also been shown to be associated with ARHI [Brant et al., 1996]. Finally, the effect of CVD on ARHI was confirmed in mouse studies [Picciotti et al., 2004]. A possible explanation for the relationship between CVD and ARHI can be sought in the occurrence of hypoxia during CDV events. Hypoxia of the cochlea may cause a reduction of mitochondrial oxidative phosphorylation, cochlear damage, and an accumulation of mitochondrial mutations, with a decreased function of the acoustic neural system and hearing loss as a consequence [Dai et al., 2004].

Bone Mineral Density

It has been suggested that a reduced bone mineral density (BMD) contributes to ARHI, although this remains controversial. In males, an inverse relation between BMD and hearing loss was found, but this relation was not observed in females [Helzner et al., 2005]. Nevertheless, in a study performed on a population of rural women, an association between ARHI and reduced BMD was detected in females as well [Clark et al., 1995]. Although no significant association could be found between estrogen supplement intake in the past and current hearing loss, hormonal changes may be the cause of the reduced BMD and ARHI in these woman [Clark et al., 1995]. None of the women were current users of estrogen supplements, and beneficial effects of estrogen on bone density is only seen among current users [Sowers et al., 1993]. Finally, a third study could not detect a relation between hearing ability and BMD [Purchase-Helzner et al., 2004]. The inconsistencies between these various studies may be caused by the investigation of BMD derived from bones that were different from the cochlear bones. Femoral neck bone contains a higher percentage of cancellous bone similar to the temporal bone, while the radius and the hip bone are less similar.

Head Trauma

Head trauma has been shown to have an effect on hearing loss [Rosenhall et al., 1993]. This may be due to the disruption of the membranous portion of the cochlea, to alterations in the microcirculation of the cochlea, or to hemorrhage into the fluids of the inner ear [Fitzgerald, 1996]. The hair cell damage due to head trauma is most pronounced at 4–8 kHz and mimics the damage caused by acoustic trauma. Generally, the hearing loss occurs immediately and recovers gradually over a 6-month period after the injury [Fitzgerald, 1996]. In a rabbit model for closed head injury, increased otoacoustic emission latencies were observed, which would indicate that cochlear damage had occurred [Danielidis et al., 2007]. After

histopathological examination of the temporal lobe and brainstem of these rabbits, multiple hemorrhagic and necrotic areas were found [Danielidis et al., 2007], indicating that both peripheral and central damage occurs after head injury.

Immune System

Dysfunction of the immune system possibly contributes to the development of ARHI. This was shown in a study using SAMP1 mice, a model for accelerated senescence, which suffer from hearing impairment and a decreased immune function. In a first study, the transplantation of bone marrow of BALB/c mice into SAMP1 mice prevented the development of immunological dysfunction and hearing loss, indicating that some types of accelerated ARHI are not caused by effects in the cochlea, but are due to hematopoietic stem cell defects and failing immunocompetent cells derived from these stem cells [Iwai et al., 2001]. The authors could rule out an autoimmune mechanism as causative factor. They suggested that pathogen-induced infections led to an impaired immune function, followed by a decline in various functions, including hearing [Iwai et al., 2003].

Diet

It has been suggested that a poor nutritional status has an effect on ARHI [Houston et al., 1999]. Caloric restriction studies in animal models have had contradictory results to date. Caloric restriction in mice does not seem to have an effect on hearing [Willott et al., 1995], while the hearing ability of rats on a caloric-restricted diet was preserved [Seidman, 2000]. In agreement with the findings of Willott et al. [1995], no significant effects of caloric restriction could be detected in rhesus monkeys [Torre et al., 2004]. However, a study by Sweet et al. [1988] suggested that the age of onset of caloric restriction may be a determining factor for the preservation of the hearing abilities at an older age. Restriction until midlife had no protective effect on ARHI, while whole-life and after-midlife restriction did have an effect on ARHI. This fact could provide an explanation for the controversial results amongst the different studies to date.

Hormones

Hormonal effects may also contribute to hearing loss. Gender differences observed in ARHI may be due to the difference in estrogen levels between males and females. In menopausal women, estrogen therapy may slow down the development of ARHI [Hederstierna et al., 2007; Hultcrantz et al., 2006]. Animal models support these findings, as mice lacking estrogen receptor β showed progressive hearing loss [Hultcrantz et al., 2006]. High levels of aldosterone within the normal clinical range may have a protective effect on ARHI [Tadros et al., 2005]. Progestin, on the other hand, may have a negative effect on the hearing abilities in aged women receiving hormone replacement therapy, and might affect both the peripheral as well as the central auditory systems [Guimaraes et al., 2006].

Socioeconomic Status

A person's socioeconomic status has been correlated with ARHI. Lower social class and a low level of education were correlated with hearing impairment. One possible explanation could be the exposure to occupational noise. Nevertheless, the effect of the socioeconomic status remains after correcting for occupational noise. Hence, higher social class and higher education may be related to life experiences that help preserve hearing abilities, while noise exposure cannot be regarded as the only causative factor for ARHI in lower social classes [Sixt and Rosenhall, 1997]. Other possible risk factors may be related to lifestyle factors like smoking and heavy drinking, which are more prevalent in lower socioeconomic classes [Poortinga, 2007]. As mentioned previously, both smoking and heavy drinking may have a negative effect on hearing.

Genetic Factors

Little is known about the genes involved in ARHI, especially in humans. The perception of sound requires complex molecular pathways and age-related changes in any component of these pathways may contribute to hearing loss. Therefore, it is expected that many genes will participate in the etiology of ARHI. However, until recently, little research effort has been put into the identification of ARHI susceptibility genes.

Mouse Models

Due to the significant similarities of the auditory system between mice and humans, mice are very useful as a model for human hearing loss [Ohlemiller, 2006]. In fact, the first ARHI genes have been identified in mice, and some of the causative genes that are being discovered in mice may be homologous to human ARHI genes. By measuring auditory brainstem responses in 80 inbred mouse strains, it was demonstrated that many strains develop age-related hearing loss, resembling human ARHI [Grat-

ton and Vazquez, 2003]. For example, the CBA/Ca, C57BL/6J and 129S6/SvEv inbred mouse strains display age-related hearing loss similar to humans. These mice show a progressive primary decline in the high frequencies followed by increasing hearing thresholds of the low frequencies [Li and Borg, 1991; Ohlemiller and Gagnon, 2004]. BALB/c mice also show a progressive high-frequency hearing loss, but with a more rapid decline in hearing loss than seen in the C57BL/6J mouse strain [Willott et al., 1998]. Moreover, the gender differences found for human ARHI were also observed in mouse models [Guimaraes et al., 2004; Henry, 2004]. The different mouse models known for age-related hearing loss have recently been reviewed by Ohlemiller [2006].

To study the genetics of age-related hearing loss in mice, Erway et al. [1993] used different inbred and F1 hybrid strains. Their results support a genetic model for recessive alleles at three different loci which contribute to the development of age-related hearing loss in mice. They demonstrated that CBA/H-T6J mice possessed none of the recessive alleles, that DBA/2J mice were homozygous for all three loci and that C57BL/6J, BALB/cByJ and WB/ReJ strains were homozygous for one of the three loci responsible for age-related hearing loss [Erway et al., 1993].

Subsequently, *Ahl1* (age-related hearing loss 1 gene) was mapped to chromosome 10 in C57BL/6J mice. A mutation in *Ahl1* causes elevated hearing thresholds in middle-aged and old mice at high frequencies [Johnson et al., 1997]. Although this mutation causes accelerated hearing loss in mice, it is currently not known whether this is responsible for human ARHI as well. Later, it was demonstrated that the *Ahl1* gene contributes to age-related hearing loss in 9 other inbred mouse strains in a recessive way (129P1/ReJ, BALB/cByJ, A/J, BUB/BnJ, C57BR/cdJ, DBA/2J, NOD/LtJ, SKH2/J and STOCK760) [Johnson et al., 2000], and that it was allelic to the modifier of deaf waddler gene [Zheng and Johnson, 2001]. Cadherin 23 was found to be the responsible gene at the *Ahl1* locus [Noben-Trauth et al., 2003]. The B6.CAST-+^{ahl} mouse was engineered to be genetically identical to the C57BL/6 mouse except for the *Ahl1* allele that originated from the CAST/Ei mouse, which has no hearing loss. B6.CAST-+^{ahl} mice are protected from early-onset hearing loss, but older animals develop hearing loss, indicating that other loci besides *Ahl1* contribute to the differences in hearing loss observed between C57BL/6 and CAST/Ei mice.

Up- and downregulation of genes have also been implicated in ARHI. For instance, Bao et al. [2005] could demonstrate that downregulation of β_2 nicotinic acetylcholine receptors contributes to age-related hearing loss in C57BL/6J mice, while upregulation of the serotonin 2B receptor was detected in the auditory system in aged CBA/CaJ mice with hearing loss [Tadros et al., 2007a]. Also, glutamate-related genes, such as pyrroline-5-carboxylate synthetase and high-affinity glutamate receptor (Slc1a3) are down- and upregulated in the ageing auditory midbrain, respectively [Tadros et al., 2007b]. This illustrates the complexity of age-related hearing loss in mice, and suggests that it may be very complex in human ARHI as well [Keithley et al., 2004].

In 2002, Johnson and Zheng [2002] detected a second locus for age-related hearing loss in mice. *Ahl2* was located to chromosome 5, and was restricted to the NOD mouse strain and NOD-related strains. Recently, a third locus, *Ahl3,* was located to chromosome 17 [Nemoto et al., 2004] using a C57BL/6J × MSM backcross, and fine-mapped to a 14-Mb region [Morita et al., 2007]. In addition to the limited number of inbred mouse strains that present with age-related hearing loss, 17 ENU-induced mouse models have been identified showing high-frequency hearing loss. These might be good ARHI models that could shed light on the underlying mechanisms leading to ARHI [Kermany et al., 2006].

How to Analyze Genetic Factors Involved in Human ARHI

There are two major approaches to identify susceptibility genes for complex disorders like ARHI. The first possible study design consists of a linkage study. This is always a family-based approach in which the cosegregation of the disease and an allele of a genetic marker at a certain locus are investigated. The second possible strategy to study complex diseases is an association study, which studies the co-occurrence of a disease and an allele of a genetic marker. Association studies can be performed in unrelated as well as in family-based samples, but the majority of studies uses unrelated samples in a case-control study design.

Both strategies analyze genetic markers. These are variants within the genome that can be analyzed in the laboratory. Frequently used genetic markers include microsatellite markers and single nucleotide polymorphisms (SNPs). SNPs are the most frequent genetic variants within the human genome, occurring on average every 300 bp. Many frequently occurring SNPs have been identified to date, and all are enlisted in a database (http://www.ncbi.nlm.nih.gov/). These SNPs and other common variants are thought to be responsible for much of the variation seen amongst individuals. Moreover, some

of them are considered to be causative for complex disorders. This is referred to as the 'common variant, common disease' hypothesis.

During the past 10–15 years, linkage genome scans have been widely used for localization of genes involved in monogenic diseases on the basis of families segregating the disease. Such a scan consists of an analysis of a limited set of microsatellite markers (300–500) or more recently, of a limited number of SNPs (2000–10000). For association studies, SNPs have always been the preferred type of polymorphism, but many more markers need to be analyzed to cover the whole genome compared to linkage studies. Until very recently, association studies were limited to the analysis of candidate genes. Nowadays however, genome-wide scans for association are feasible. This is due to improved technology, and more specifically to the development of microarrays enabling the analysis of up to 500000 SNPs on a single array. Because of rapidly evolving methodologies for SNP genotyping and statistical analysis, coupled to a reduction in cost per genotype, nowadays SNPs increasingly become the first-choice genetic marker for linkage as well as association studies. In October 2002, the HapMap project was started (http://www.hapmap.org), which intended to map human variation taking into account linkage disequilibrium between neighboring SNPs. Several studies have demonstrated that the HapMap data are also useful for other closely related populations [Montpetit et al., 2006; Willer et al., 2006]. As such, the HapMap database has become a useful tool for effective SNP selection.

For linkage analysis of complex diseases, usually non-parametric analysis methods are preferred. This means that no assumptions about the mode of inheritance, the disease frequency or other parameters are being made. A large collection of families is a prerequisite for linkage studies. A problem that presents itself when using linkage approaches to study late-onset disorders like ARHI is the collection of families, because parents are often deceased. Therefore, very few linkage studies have been performed for ARHI to date. Within the Framingham cohort, audiometric data were collected from parents during a first phase (1973–1975) and from their children in a second phase (1995–1999). Analysis of these data resulted in linkage for ARHI at six different loci on 4 chromosomes [DeStefano et al., 2003]. In a second genome-wide linkage analysis for ARHI, a seventh locus was identified which coincided with the *DFNA18* locus [Garringer et al., 2006]. Genome-wide scans for complex diseases require replication in independent sample sets. Because so far only two genome-wide scans have been performed for ARHI, there is still a lot of work to be done before the genetic basis of ARHI will be clarified.

Association studies for ARHI can be performed in two ways. ARHI can either be described as a dichotomous trait, where a SNP allele, which confers susceptibility to a disease, is expected to occur more in affected individuals (cases) compared to the unaffected group (controls). A disadvantage of treating ARHI as a dichotomous trait (affected/unaffected) is the loss of statistical power, as generally information is lost when a quantitative trait such as ARHI is dichotomized [Page and Amos, 1999]. Fransen et al. [2004] proposed the calculation of a Z-score to enable treatment of ARHI as a quantitative trait. This Z-score is an age- and gender-independent value, based upon the ISO 7029 standard [Fransen et al., 2004]. Using this approach, random samples genotyped for a particular SNP in a candidate gene can be grouped according to their genotype and subsequently statistically analyzed using ANOVA-based methods.

Although genome-wide association studies are feasible, they are still very expensive. Up to now only association studies on functional candidate genes have been published. These candidate genes are selected based on biological and physiological information and the biochemical pathways in which they are acting. Genes causing monogenic forms of a disease are obvious candidate susceptibility genes for the complex forms of the disease [Tabor et al., 2002].

Previous association studies performed for ARHI have investigated such candidate genes. For example, Van Laer et al. [2002] analyzed *DFNA5,* a gene causing autosomal dominant hearing loss, but no association could be detected. Ates et al. [2005] performed a case-control study to test the hypothesis that glutathione-related antioxidant enzyme levels were associated with the risk of ARHI, but they could not detect an association. An association study analyzing N-acetyltransferase 2 *(NAT2),* a carcinogen-metabolizing enzyme, as a candidate gene for ARHI resulted in a significant association for the *NAT2**6A polymorphism and ARHI [Unal et al., 2005]. In addition, association with ARHI was found for different SNPs within a 13-kb region in the middle of *KCNQ4,* a gene for autosomal dominant hearing loss, in two independent sample sets [Van Eyken et al., 2006].

Do Mitochondrial Mutations and Reactive Oxygen Species Contribute to ARHI?

Mitochondrial mutations cause diseases typically seen in the elderly [Wallace, 1997]. Different mouse models for ageing in general have indicated that the accumulation of

mitochondrial DNA mutations might contribute to ARHI [Kujoth et al., 2005; Trifunovic et al., 2004; Zhang et al., 2002]. Indeed, in patients with ARHI, a highly significant increase in mitochondrial mutations in auditory tissue has been demonstrated [Fischel-Ghodsian et al., 1997]. The acquired mitochondrial mutation that occurs most frequently in humans is the mitochondrial deletion mtDNA^{4977}, which deletes 4977 bp between two 13-bp repeats starting at nucleotides 8470 and 13447. Analyses of human temporal bones indicated that mtDNA^{4977}, the so-called 'common' deletion, occurred frequently in ARHI patients [Bai et al., 1997; Dai, 2004], while it was almost absent in age-matched control patients without a history of ARHI [Seidman et al., 1996]. Similar analyses were conducted in rats, where a 4834-bp deletion was associated with ARHI and ageing in general [Seidman et al., 1997].

Cells that accumulate large numbers of mitochondrial mutations become bioenergetically deficient [Seidman et al., 2002a], with cell loss as a consequence. This is mainly a problem in postmitotic tissue, like the inner ear, where no regeneration of cells is possible, resulting in a permanent loss of sensory cells. Mitochondrial metabolites, which upregulate mitochondrial function and improve energy-producing capabilities within the cell, have been shown to delay the progression of cellular losses and ARHI [Seidman et al., 2000].

Reactive oxygen species (ROS), like the superoxide anion, hydroxyl and peroxyl, are produced both in healthy and diseased state and are controlled by antioxidant defense mechanisms. Although these repair mechanisms exist, an imbalance between production and removal of ROS can occur, with oxidative stress as a consequence [Evans and Halliwell, 1999]. While investigating oxidative stress in the cochlea of ageing CBA/J mice, Jiang et al. [2006] detected different ROS and time-specific oxidative changes in the different tissues of the ageing cochlea. Actions of free oxygen radicals may cause genetic and cellular alterations preceding cell dysfunction with senescence as a consequence [Seidman et al., 2002a]. As such, ROS have been implicated in ARHI by several lines of evidence. For example, in 24-month-old Fisher rats, the glutathione level is reduced by 86% in the auditory nerve [Lautermann et al., 1997]. As glutathione is a scavenger for ROS, this may increase the concentration of free radicals in the inner ear. In addition, Unal et al. [2005] detected an association for N-acetyltransferase and ARHI in humans. N-acetyltransferases are known to contribute to the detoxification process of exogenic compounds and the protection against ROS by N-acetylation or O-acetylation of the toxic compounds.

The superoxide anion is the most common ROS. It causes auditory sensory cell damage, eventually resulting in apoptosis of auditory neurons and hair cells [Huang et al., 2000]. Superoxide dismutases (SODs) form the first line of defense against cochlear damage caused by superoxide anion [Coling et al., 2003]. Under normal circumstances, copper/zinc superoxide dismutase (SOD1) is highly expressed within the cochlea. SOD1 deficiency in mice induces cochlear hair cell degeneration and loss of spiral ganglion cells and nerve fibers, with ARHI as a consequence [McFadden et al., 1999a, b, 2001]. Lecithin, a polyunsaturated phosphatidylcholine responsible for SOD activation, has a protective effect and preserves the hearing abilities in ageing subjects [Seidman et al., 2002b].

Therapies

The only intervention currently available for subjects with ARHI is a hearing aid. Although hearing aids can improve the hearing ability of affected individuals, they are only suitable for a limited number of people. This is mainly due to the limited efficacy in improving speech understanding, especially in noisy environments. Future therapies for ARHI might rely on basic rather than on symptomatic approaches. This requires a better understanding of the molecular and cellular processes taking place in the inner ear, which will demand further research efforts.

One of the possible future strategies is gene therapy. Recently, a very promising gene therapeutic experiment has been conducted, which raises hopes for the future. By the introduction of *Math1,* a gene that is important in hair cell development in the cochlea, regrowth of hair cells has been obtained. Most interestingly, the recovery of hearing abilities could be demonstrated in mice [Izumikawa et al., 2005].

Many different administration routes for gene therapy have been suggested, such as infusion with osmotic minipumps, direct microinjection into the cochlea and application of a vector-transgene complex-soaked Gelfoam directly onto the round window [Lalwani et al., 2002]. Similar approaches can also be used to administer pharmacological substances into the inner ear [Wang et al., 2002], such as antioxidants [Lefebvre et al., 2002] and growth factors [Bowers et al., 2002; Lefebvre et al., 2002; Malgrange et al., 2002].

Due to the identification of ARHI susceptibility genes new leads for pharmacological intervention may be dis-

covered. To administer pharmacological substances, two strategies can be used: systemic administration, and local therapy. Systemic therapy requires high doses of the drug, which might lead to toxic high concentrations, while due to the isolated localization of the cochlea, high drug concentrations can be obtained within the inner ear by means of local therapy. However, a disadvantage of local therapy is the fact that it is invasive and potentially harmful for the cochlea. In the future, this may be circumvented by new and safer administration routes such as described above.

Another treatment strategy under development is the implantation of stem cells. Ito et al. [2001] demonstrated that neural stem cells survive when grafted into newborn rat cochleae. These neural stem cells adopted the morphologies and positions of hair cells and were well adapted to the environment of the cochlea [Ito et al., 2001]. Also, stem cells and embryonic neurons transplanted in the inner ear have been shown to survive, migrate, differentiate and extend neurotic projections in the auditory system of adult mammals [Hu and Ulfendahl, 2006]. Rivolta et al. [2006] succeeded in the generation of inner ear progenitor cells from murine embryonic cells, which were subsequently differentiated into hair cells and potentially also into other inner ear cell types. Out of all these efforts, a new treatment for ARHI may arise in the future.

Conclusion

Despite the growing interest in ARHI as a complex disease, currently little is known about the genetic factors contributing to the disease. Up to now, the emphasis has been on the investigation of the contribution of environmental factors. Although there is still some controversy about some of these, the results obtained for environmental factors start to contribute to the formulation of prevention strategies for ARHI.

The first steps to elucidate the genetic factors involved in ARHI have also been made. Genome-wide linkage studies have resulted in seven candidate susceptibility regions for ARHI [DeStefano et al., 2003; Garringer et al., 2006]. Association studies have revealed the first two genes involved in ARHI [Unal et al., 2005; Van Eyken et al., 2006]. The newly found genetic interest in ARHI will result in the identification of several susceptibility genes in the coming years. This will surely have an impact on our molecular genetic knowledge of the disease, and will, hopefully, in the long term lead to the development of new treatments. As the overall population is still ageing, and as hearing loss is the most common sensory impairment affecting the elderly, these new therapies will surely contribute to a better quality of life and improved economic productivity for an increasing number of people.

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