Chapter 14
Genetics and Age-Related Hearing Loss

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Abbreviations

ABR	Auditory brainstem response
ARHL	Age-related hearing loss, presbycusis
AVCN	Anteroventral cochlear nucleus
DCN	Dorsal cochlear nucleus
PCR	Polymerase chain reaction quantitative measure of gene expression
PTS	Permanent threshold shift, permanent hearing loss
PVCN	Posteroventral cochlear nucleus
ROS	Reactive oxygen species, free radicals
SGN	Spiral ganglion neuron, auditory nerve fiber
SNP	Single nucleotide polymorphism

14.1 Introduction

Age-related hearing loss (ARHL)—presbycusis—is the most common communication disorder and neurodegenerative condition of our aging population; and one of the top three chronic medical conditions, along with arthritis and cardiovascular diseases. Mild to moderate ARHL can negatively impact social communication, family relations, and professional productivity, particularly when speech communication is

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required in noisy environments or multi-talker listening situations (Frisina and Frisina 1997). Presbycusis can lead to social/familial isolation and psychological problems such as depression, anxiety, and/or distress. ARHL is often accompanied by tinnitus—ringing of the ears—i.e., perception of phantom sounds in the absence of external acoustic stimuli, which worsens the impact of presbycusis (Rauschecker et al. 2010). There are currently no medical treatments to prevent or decrease the symptoms of ARHL. Therefore, a better understanding of the genetics of ARHL would likely pave the way for novel biomedical therapeutics including development of pharmacological or other forms of interventions. Limited advances have been made concerning the genetics of ARHL for human genes whereas genetic variations in animal models have been fundamental in advancing our knowledge of the neurological consequences of ARHL. Hopefully, increasing information on candidate genes from animal studies will define a path to increase our knowledge of genetics of ARHL in humans. This chapter will summarize current knowledge of genetics of presbycusis in animal models, and emerging evidence on genes related to ARHL in humans.

14.2 Genetics of ARHL in Animal Models

There is a rich history and scientific literature built on animal models, mostly rodents, for understanding normal auditory system function, and what goes awry in cases of ARHL. A wealth of genetic information discovered through use of transgenic and knockout animals have made mice a valuable model in biomedical research. Part of this literature has involved the use of genetically engineered rodent strains, in particular, inbred strains of mice with interesting auditory aging characteristics (Henry 1982; Erway et al. 1993; Johnson et al. 2006). In some cases, as presented below, inbred mouse strains with accelerated ARHL have emerged, which allow for isolating different perceptual and biological characteristics of ARHL; and in a few instances, genes that cause ARHL in mice have been found in certain human populations.

14.2.1 Utilization of Inbred Mouse Strains

Pioneering genetic studies in animal models have yielded valuable insights into the underlying mechanisms responsible for ARHL. For example, one of the most useful mouse strains in this regard has been the C57 (Henry 1982). One of the key components of the electromechanical transduction apparatus of mammalian hair cells, which is a significant part of our mechanism for hearing, are the tip links on the dorsal portions of the stereocilia residing on the top of cochlear inner and outer hair cells (Sotomayor et al. 2010). When sound enters the cochlea, the basilar membrane vibrates, causing a shearing force at the top of the hair cells as they vibrate along

with the surrounding organ of Corti. This sound-induced shearing force opens and closes potassium channels in the stereocilia tip links extremely rapidly, controlling depolarization of the hair cells in response to acoustic stimulation. These hair cell tip links are composed of two proteins, protocadherin-15 and cadherin-23, wherein mutation of the latter's gene causes progressive hearing loss or deafness in certain circumscribed human populations (Astuto et al. 2002; Noben-Trauth et al. 2003; Schultz et al. 2005). The C57 and BALB mouse strains have a mutation in the *CDH23 Ahl* gene, resulting in production of a cadherin-23 protein that is functionally defective, therefore interfering with the flow of potassium ions into the hair cells from scala media. The presence of this defective protein results in an age-accelerated, high-frequency hearing loss in C57, BALB/c, and certain other mouse strains (Johnson et al. 1997, 2000; Willott et al. 1998; Keithley et al. 2004; Zheng et al. 2009), accompanied by significant loss of hair cells (Spongr et al. 1997; Jimenez et al. 1999). For example, the typical C57 mouse has a high-frequency hearing loss of the same magnitude as an elderly human, by the time the mouse is 6 months old. By the time the C57 reaches middle-to-old age, with old age being about 24 months for mice, these mice are profoundly deaf. The DBA mouse strain, which has additional copies of the mutated *CDH23* gene, has an even more rapid ARHL than the C57 strain.

In some sense, a 6-month-old C57 or DBA mouse has an "old" cochlea, but still a "young adult brain," allowing for isolation of certain key aspects of ARHL. For instance, when considering ARHL broadly, changes of hearing with age could be the result of *direct aging of the cochlea*; *direct aging of the parts of the brain used for hearing*—central auditory system; or, they could be linked to age-related plasticity of the central auditory system *due to reduced inputs from the aging cochlea*. This latter phenomenon is sometimes referred to as peripherally induced central effects (Frisina et al. 2001) or peripherally induced brain plasticity. One research strategy has been to compare changes in the central auditory system of aging C57, BALB, and DBA, with other mouse strains, for example, the CBA mouse strain, which loses its hearing more slowly with age like most humans, to tease out direct aging effects from peripherally induced central plasticity with age (e.g., Kazee et al., 1995).

James Willott and colleagues performed many of the pioneering investigations taking full advantage of the rapid peripheral hearing loss of C57s to explore age-induced auditory neuroplasticity. They discovered significant reorganization of the tonotopic maps at key levels of the central auditory system (Willott 1986, 1991; Willott et al. 1988). In the normal mammalian central auditory system, each of the key nuclei, such as the cochlear nucleus, inferior colliculus (auditory midbrain), and auditory cortex, are tonotopically, or cochleotopically organized. This means that high-frequency sound information, from the cochlear base, is represented in one spatial region of each central auditory center; low-frequency information from the cochlear apical turn is represented in a different region; and middle-frequency sounds are processed systematically in a spatial area in between the high and low frequencies. Willott and coworkers discovered that the high-frequency portions of these central auditory maps were rewired to process lower frequency information as the C57 mice aged. Specifically, in the inferior colliculus, ventral areas that normally

process high sound frequencies became increasingly more responsive to lower frequencies as the C57s aged, and their basal cochlea became increasingly dysfunctional. This demonstrates neural plasticity, or axonal sprouting, where the terminal endings of lower frequency neurons migrate into central auditory regions that previously processed higher frequencies.

Consistent with this functional reorganization of the central auditory system in response to reduced high-frequency inputs (declines in high-frequency outputs from the cochlear base), Willott and colleagues observed underlying structural changes (Willott et al. 1985, 1987). Specifically, for aging C57 spiral ganglion neurons (SGNs) and in the anteroventral cochlear nucleus (AVCN), neuron packing density and cell number *decrease* between 1 and 7 months; whereas these values are stable in CBA mice that lose their hearing slowly with age. The AVCN C57 cell declines were most pronounced in the dorsal area of the AVCN where high frequencies are normally coded in the mammalian ventral cochlear nucleus tonotopic map. Consistent with the central plasticity findings of Willott and colleagues, it was also discovered that the efferent feedback system, from the superior olivary complex back to the cochlear hair cells (MOC system), declines much more rapidly with age in C57s compared to CBAs (Frisina et al. 2007).

14.2.2 Excitotoxicity in C57 and CBA Genetic Strains

One of the leading theories of neurodegenerative causation involves neural degeneration due to repetitive use throughout life, sometimes referred to as *excitotoxicity*. This can result in the buildup of reactive oxygen species (ROS), a by-product of energy production from conversion of glucose and oxygen to energy (ATP), ROS, and water in mitochondria. Excitotoxicity can also invoke the undesired buildup of calcium ions (Ca^{++}) inside neurons, since Ca^{++} enters the neuron during synaptic vesicle release. This occurrence is often referred to as *Ca^{++} excitotoxicity* and can be somewhat regulated by changes in concentration of intracellular Ca^{++} regulatory peptides such as calbindin, parvalbumin, and calretinin. Frisina, Zettel, and colleagues have investigated these changes in two of the most useful mouse strains for studies of ARHL, the C57 and CBA. Some noteworthy findings include age-linked declines in calbindin in key nuclei of the central auditory system, such as in the superior olivary complex of C57s and the auditory midbrain of both strains (O'Neill et al. 1997; Zettel et al. 1997). Interestingly, Zettel and coworkers discovered an *activity-dependent upregulation* of calretinin in the CBA inferior colliculus in old CBAs, which was abolished by deafening CBAs as young adults, then allowing them to live to old age (Zettel et al. 2001).

Barbara Canlon's group has also made some of the key discoveries in the area of age-related changes in key calcium-binding proteins in the auditory brainstem, focusing on calbindin, calretinin, and parvalbumin. They utilized a stereological method, the optical fractionator, to determine the total number of neurons and the number of immunostained neurons in the posteroventral and dorsal cochlear nuclei

(PVCN and DCN) (Idrizbegovic et al. 2001, 2003, 2004). Specifically, they found that in aged C57s and BALB mice, there was a significant age-linked decline in the number of neurons, but surprisingly, the number of parvalbumin-stained neurons *increased* with age (C57s only), as did the percentage of neurons showing calbindin (C57s only), calretinin, and parvalbumin staining in the older mice. In addition, correlations were found between peripheral cochlear pathology, e.g., significant loss of hair cells and SGNs with age, and the age changes in the Ca^{++}-binding proteins. In aging CBAs, similar but less dramatic total neuron declines and relative *increases* in parvalbumin and calbindin were found on a much slower time frame in the PVCN and DCN. Interestingly, in the Long Evans rat strain, which lose hearing slowly like CBA mice and most humans, an *upregulation of parvalbumin* was observed in the aging inferior colliculus (Ouda et al. 2008). Taken together, these studies of the aging brainstem auditory system indicate a relative *upregulation* of calcium-binding proteins with age in the cochlear nucleus and inferior colliculus of several strains of mice and rats. These data indicate that calcium-binding proteins act as endogenous protective agents for reducing the aging effects of Ca^{++} excitotoxicity and/or compensate for reductions in cochlear inputs. It could be that the brainstem auditory neurons expressing these Ca^{++} regulatory proteins are *more likely to survive with age*.

Utilizing a copper/zinc superoxide dismutase (Cu/Zn SOD) knockout mouse strain, McFadden and colleagues (1999a, b) shed light on possible roles of antioxidants in slowing down ARHL. Cu/Zn SOD comprises a first-line defense against age-linked free radical (ROS) damage in the cochlea and other tissues. McFadden and coworkers compared auditory sensitivity (ABRs), and hair cell and SGN counts for homozygous, heterozygous, and 129/CD-1 control mice with both copies of the normal *Cu/Zn SOD* gene at 13 months of age (mouse middle age). They found that the homozygotes and heterozygotes showed significant ABR threshold elevations and hair cell and SGN loss, with phenotypic variability the highest in the heterozygotes. They concluded that Cu/Zn SOD deficiencies *increase the vulnerability of the aging cochlea* to damage, most likely through deficits in metabolic pathways that normally scavenge superoxide radicals.

14.2.3 Interactions Between the Genetics of ARHL and Environmental Ototoxicities

Another fascinating use of the different rodent genetic strains has shed light on relations between noise-induced hearing loss and aging. In the field of hearing research, an important question has been: Are aging animals more susceptible to noise exposure than young adults? Ohlemiller and colleagues (2000, 2006) utilized the CBA, C57, and BALB strains to examine this question. Their main findings were that for young adults, BALB mice were most susceptible to noise damage, followed by C57s, then CBAs. These investigators concluded that the effects of the *Ahl* gene mutation that accelerates ARHL may also weaken the cochlea for tolerating a damaging noise exposure. Interestingly, the middle age mice of each of these three

strains were *less vulnerable* to noise damage relative to their younger counterparts of the same strain. On a related topic, Kujawa and Liberman (2009) exposed young adult CBA mice to robust, sustained, sub-noise-damage-threshold levels of loud noise. They then allowed the CBAs to age otherwise normally from 2 months to up to 2 years, tested the hearing of these mice, and sacrificed them for cochlear anatomical studies. They discovered that the noise-exposed mice had normal hearing thresholds, but a greater degree of suprathreshold ABR amplitude suppression, much more hair cell/auditory nerve ribbon synapse cochlear structural damage, and greater loss of SGNs, relative to age- and strain-matched control mice. Their conclusion was that *prolonged*, *subthreshold noise exposures can accelerate certain key aspects of ARHL*, *including SGN neurodegeneration*.

Similar to relations between loud noise exposure and ARHL, interactions for ototoxic antibiotics and presbycusis have been explored. Jochen Schacht and colleagues examined interactions of aminoglycoside ototoxicity in adult CBA, C57Bl, and BALB mice, and Sprague–Dawley rats (Wu et al. 2001). The young adult mice and rats were injected subcutaneously twice/day with kanamycin for 2 weeks. This resulted in ABR threshold shifts of 50–70 dB in the 20–24 kHz range, as well as hair cell loss and vestibular/balance deficits. The strain effects were similar to the noise study presented above, in that the BALB strain was most susceptible, but different in that the CBAs were next, with the C57s showing the smallest of the effects, along with the rats. Lastly, Schacht and coworkers found that administration of the antioxidant 2,3-dihydroxybenzoate could attenuate the kanamycin-induced auditory threshold shifts, and there was a relation with the amount of melanin in the cochlear lateral wall to this antioxidant protection; in this case, more was better.

14.2.3.1 Genetics of Cochlear Age-Related Hearing Loss

In the largest gene expression study of the aging auditory system to date, human or animal model, Frisina, Zhu, D'Souza, and colleagues assessed gene expression changes in the CBA aging cochlea and auditory midbrain. Here, the auditory phenotype (ABR, DPOAE) was measured in 40 CBA mice of different ages, prior to sacrifice. Next, the cochlear duct (organ of Corti, hair cells, lateral wall) and inferior colliculus were dissected and saved for each mouse (the samples from different mice were *not* combined). Then, these 80 samples (40 cochlea, 40 inferior colliculus) were applied to 80 Affymetrix mouse gene chips, which allowed assessment of over 15,000 mouse genes, for each of the 40 subjects of this study. Having gene expression and phenotypic hearing measures on each mouse, allowed for quantitative assessment of the relations between genotype and phenotype. Any genes reported to change with age and hearing loss in the microarrays were verified with quantitative PCR follow-up with portions of the same tissue sample extracts that were utilized for the gene chips. These experimenters examined families of genes that are important in the areas of age-related neurodegeneration and aging sensory systems. Specifically, transcriptional gene expression patterns of 318 apoptosis-related genes were analyzed. Thirty eight probes (35 genes) showed significant age

differences in expression. These gene families included Caspases, B-cell leukemia/lymphoma 2 family, P53, Calpains, Mitogen-activated protein kinase family [MPK], Jun oncogene, Nuclear factor of kappa light chain gene enhancer in B-cells inhibitor-related and tumor necrosis factor-related genes [NFκB] (Tadros et al. 2008). The GeneChip results of 31 genes were validated using the new TaqMan Low Density PCR Array (TLDA). Eight genes showed highly correlated results with the GeneChip microarray data. These genes were: activating transcription factor 3, B-cell leukemia/lymphoma 2, Bcl 2-like 1, caspase 4 apoptosis-related cysteine protease 4, Calpain 2, dual specificity phosphatase 9, tumor necrosis factor receptor superfamily member 12a, and Tumor necrosis factor superfamily member 13b, suggesting they may play critical roles in inner ear aging.

This research group also analyzed changes in immune system genes with age in this overall study. Several strategies for relating these changes to pathway analysis and other innovative biostatistical analyses revealed immune response pathways involved in cochlear ARHL (Xiao et al. 2004; Tra et al. 2011). Specifically, analyses showed that B cell-mediated humoral immune function plays a role in the underlying etiology of presbycusis, similar to certain other neurodegenerative diseases. Other top pathways identified included those involving dendritic antigen-presenting cells, carbohydrate binding, G-protein coupled receptor binding, and epithelial-to-mesenchymal transition pathways.

Donald Caspary and colleagues have previously demonstrated through an elegant series of investigations in the rat auditory brainstem that an age-linked down-regulation of the primary inhibitory neurotransmitter systems takes place, namely GABA and glycine (Ling et al. 2005; Caspary et al. 2008; Wang et al. 2009). Examination of the GABA system in the mouse cochlear gene array study also yielded some new information on this matter (D'Souza et al. 2008). Specifically, expression of GABA-A receptor subunit α6 was *upregulated* with age and hearing loss, whereas subunit α1 declined. Additionally, GABA-A receptor associated protein like-1, and GABA-A receptor associated protein like-2 genes were significantly *downregulated* with age and hearing impairment in the CBA mouse cochlea.

In the antioxidant family the glutathione peroxidase 6, *Gpx6* and Heat shock protein 1, *Hspb1* genes were found to be *upregulated* with ARHL, and the thioredoxin reductase 1, *Txnrd1* gene was *downregulated* in the CBA mouse cochlea (Tadros et al. 2014). These ARHL antioxidant gene expression discoveries open the door for future interventions, where gene expression patterns can be examined and manipulated clinically to reduce pathologies of ARHL at both peripheral and central levels. These biomedical interventions could involve novel pharmacological or gene therapy approaches.

Aquaporins, particularly aquaporin 4 (coded by *Aqp4*), are membrane proteins critical for the movement of water and ion flux across cell membranes, including cells of the inner ear and central auditory system. It was found that in the cochlea *Aqp4* gene expression *declined* with age and hearing loss, and in the inferior colliculus, an initial down-, then upregulation in old age was discovered (Christensen et al. 2009). The authors theorized that these changes in *Aqp4* gene expression represent an age-related disruption of ion flux in the cochlear fluids that are responsible for

ionic gradients important for sound transduction in cochlear hair cells necessary for hearing. In regard to central auditory processing, aquaporin gene expression age changes may affect neurotransmitter cycling involving supporting cells, thus impairing complex sound neural processing with age.

14.2.3.2 Genomics of the Aging Auditory Brain

Serotonin (5-HT) is a monoamine neurotransmitter that modulates neuron discharges in the cochlea, inferior colliculus, and auditory cortex. Specific functions of serotonin exert themselves via specific receptors; and one of those is the serotonin 2B receptor. As another component of the overall CBA gene expression investigation, Tadros and colleagues (2007a), discovered that the serotonin 2B receptor gene was *upregulated* with age in both cochlea and inferior colliculus, and was correlated with functional hearing phenotypic measures. Additional immunohistochemical protein expression studies of inferior colliculus neurons revealed the presence of more serotonin 2B receptors in old CBA mice relative to young adults, particularly in the external nucleus. So, in this case, the gene and protein expressions both increased with age, perhaps to compensate for less serotonin transmitter release in the aging auditory system.

Glutamate is the primary excitatory neurotransmitter in both the peripheral and central auditory systems. Gene expression changes of glutamate and glutamate-related genes may be a key factor in the pathogenesis of ARHL. Tadros and colleagues, as part of the overall CBA mouse microarray investigation, examined age changes for glutamate-related genes in the inferior colliculus (Tadros et al. 2007b). Gene expression of 68 glutamate-related genes was explored using both the microarrays and real-time PCR. Two genes showed consistent differences linked to ARHL: Pyrroline-5-carboxylate synthetase enzyme (*Pycs*) showed *downregulation*, and a high-affinity glutamate transporter (*Slc1a3*) showed *upregulation* with age and hearing loss. Since Pycs helps convert glutamate to proline, its deficiency in old age may lead to both glutamate increases and proline deficiencies in the auditory midbrain, perhaps potentiating glutamate toxicity and a loss of proline neuroprotective effects with age. The upregulation of the *Slc1a3* gene may reflect a cellular compensatory mechanism to protect against age-related glutamate or calcium excitoxicity.

14.2.4 Altering the Time Course of ARHL in Mouse Strains: Interventional Therapeutics

Auditory neuroscientists are starting to test drug or acoustic interventions to alter the course of presbycusis in different mouse strains. Statin drugs have greatly improved the treatment of hypercholesterolemia by inhibiting cholesterol biosynthesis. Their immunomodulatory and anti-inflammatory actions and beneficial

effects on treatment of atherosclerosis are significant. Atorvastatin is one such drug, and Syka and colleagues (2007) administered it to C57Bl/6J mice (10 mg/kg/day in chow diet) for 2 months. The treated mice showed larger amplitudes of distortion product otoacoustic emissions (DPOAE) relative to the non-treated control group, indicating improved survival of outer hair cell function in the cochlea of aging C57Bl/6J mice. They also observed decreased expression of intercellular and vascular adhesion molecules in the aortic wall of the atorvastatin-treated mice, suggesting that the atorvastatin *reduces* endothelial inflammation, thereby improving the inner ear blood supply, and slowing down certain presbycusic mechanisms.

Willott, Turner, and colleagues (Willott and Turner 2000; Willott et al. 2000) pioneered investigations of acoustic modulation of the progression of presbycusis in various mouse strains. Initially, they exposed young adults with accelerated ARHL, including BALB/c mice, to a 70 dB broadband noise for 12 h/day for at least 1 month. They measured ABR audiograms and pre-pulse inhibition of the acoustic startle response to assess the effects of the augmented acoustic environment (AAE) on the time course of ARHL. Relative to the CBA strain, which showed no benefit, the AAE improved ABR audiogram thresholds and PPI responses in the BALB/c and other rapid-ARHL strains, relative to unexposed control mice of the same age and strain. These investigators noted that the beneficial AAE effects did not occur if the onset of the AAE was too late, i.e., after the severe hearing loss had taken place in the BALB/c mice.

Follow-up studies by Turner, Willott, and others revealed substantiating evidence that an AAE can delay key functional and structural aspects of ARHL in the mouse strains with rapid presbycusis, including C57s and DBAs (Willott and Turner 1999; Willott and Bross 2004; Willott 2009; Willott et al. 2010). In addition, structural improvements induced by the AAE were uncovered in DBAs, including preservation of hair cells and AVCN neurons, and *reduction* of AVCN volume declines with age (Willott et al. 2005). Turner and colleagues (2013) also exposed old (22–23 months) CBA mice to AAEs for 6 weeks, and found structural and functional benefits for males, but not females, suggesting influences of hormonal differential effects late in life. Lastly, Michael Kilgard and colleagues have demonstrated that the beneficial effects of AAEs can manifest themselves at the level of auditory cortical neurons, both anatomically and physiologically in Sprague–Dawley rats (Engineer et al. 2004; Bose et al. 2010).

14.3 Human Genetics of ARHL

Perhaps surprisingly, much less is known about human genes involved in ARHL, relative to the animal model research literature. This is fundamentally due to the fact that older people can have a number of environmental factors that impact negatively on their hearing abilities in old age, unlike laboratory rodents that live in a relatively pristine environment, healthwise. These human environmental variables, such as loud noise (see Chap. 7 by Altschuler), health factors such as

cardiovascular disease, diabetes, and smoking (see Chap. 4 by Shirwany and Seidman), occupational exposure to heavy metals (see Chap. 5 by Park), side effects of chemotherapeutic agents (see Chap. 11 by Laurell) or antibiotics (see Chap. 10, by Rybek), nutrition (see Chap. 6 by Spankovich), and aging factors themselves, *can operate independently or in concert with human genetics*, to determine hearing abilities by the time humans reach old age. These environmental hearing loss variables, which are largely unknown or not accurately reported on health-history or hearing health patient/subject questionnaires, greatly complicate the abilities of hearing researchers, human geneticists, and epidemiologists to tease out environmental effects from human genetic etiologies for ARHL. In addition, within the fields of biostatistics, genetics, and epidemiology, there is great debate about the statistical methodologies and bioinformatics tools that are appropriate and valid for separating environmental factors from genetic causation in human studies of aging. Indeed, disagreements about even the number of subjects needed to definitively demonstrate the association between a human gene and an age-linked neurodegenerative disorder like presbycusis is up for vigorous debate, and is quite dependent on experimental design, number of hearing tests involved in the study, and subject selection and screening criteria. So in sum, very little is known about the human genetics of ARHL, with only one gene strongly associated, as explained next.

14.3.1 Pioneering Epidemiological Studies Indicate That ARHL Is Heritable

The Framingham epidemiological study of human aging had some simple hearing measures as part of its experimental design, including pure-tone, audiometric thresholds. Gates and colleagues (1999) analyzed these data in detail and found correlations between the degree of ARHL among family members, such as old parents and their middle age children, as well as among siblings. Interestingly, it was found that a middle age child's degree of ARHL was more related, on the average, to their mother's degree of hearing loss, compared to their father's severity of presbycusis. Since mitochondria are the only cellular organelles that have their own DNA (genes), and we receive all of our mitochondria originally from our mothers' egg, the stronger correlation between mothers and their children for ARHL, indicates involvement of some mitochondrial genes in severity of presbycusis. These Framingham findings have been confirmed in part in other subsequent studies of human hearing and aging (e.g., Karlsson et al. 1997; Christensen et al. 2001; Viljanen et al. 2007), and together they strongly implicate genetic factors in the etiology and progression of presbycusis, with heritability estimates varying between 0.25 and 0.75. With this knowledge in hand, hearing researchers, statisticians, and epidemiologists have begun the quest for identifying human genes that cause, or predispose one to ARHL, which is the topic of the next section of this chapter.

14.3.2 Initial Investigations Are Suggestive but Inconclusive

Huyghe et al. (2008) conducted a cross-sectional family-based study using audiometric data. They utilized principal component analysis, were able to reduce the dimensionality of the multivariate audiometric phenotype and still capture much of the variation, and retained biologically important characteristics of the audiograms. In addition, they carried out a genome-wide association and a linkage scan with high-density single nucleotide polymorphism (SNP) microarrays. No association signals reached genome-wide significance, but linkage analysis yielded a linkage peak at 8q24.13–q24.22, this area of interest was found on chromosome 8, and variation in this region of chromosome 8 was related to audiogram shape in the elderly. Specifically, genetic variations of chromosome 8 were linked to the degree of either: high-frequency hearing loss slope, or degree of concavity of the audiogram shape (the degree of improvement of thresholds at high frequencies). The 8q24.13–q24.22 signal reached genome-wide significance, as assessed by simulations, representing the first locus for an ARHL-related trait. Additional candidate gene association studies have been published since then, as reviewed in Van Eyken et al. (2007), without conclusive identification of a presbycusis gene.

14.3.3 GRM7 *and Human Presbycusis*

Glutamate is the primary excitatory neurotransmitter for synaptic transmission between inner ear hair cells and nerve fibers of the eighth cranial nerve. The *GRM7* gene codes for one of the key proteins of the glutamate receptors on SGNs that synapse with hair cells. Any abnormalities or variations in the normal structure of GRM7 receptor protein could result in distorted information being transmitted to the central auditory system by the SGNs, and hearing impairment, including ARHL. Friedman and An International Group of Colleagues (2009) conducted a 500K-SNP genome-wide association study of a large European cohort of older subjects (Study 1). They identified one haplotype within the *GRM7* gene (OMIM ID: 604101) as harboring a significant risk allele for presbycusis. Friedman and coworkers also reported another, smaller experiment (Study 2), in which the locus was more finely mapped and confirmed in a second European group of subjects. Although this is the first human presbycusis gene to reach genome-wide significance, limitations of their genetic analysis methods using *Z* scores, either a single intronic SNP or the adjacent haplotype block, allowed for some inconclusiveness in their findings.

To seek more definitive conclusions regarding the role of the *GRM7* gene in human presbycusis, Newman, Frisina, Friedman, and their colleagues undertook a further investigation of *GRM7* haplotype block variations in a group of 687 North American subjects over the age of 58 (mean age = 71 years), who underwent the most extensive battery of hearing tests of any study of the genetics of human presbycusis (Newman et al. 2012). This investigative team treated these auditory test

data as quantitative, continuous variables, and employed mixed modeling analyses to explore the relationships of *GRM7* haplotypes and SNP genotypes. They found that *GRM7* alleles are strongly associated primarily with peripheral measures of hearing loss, particularly pure-tone and speech reception thresholds in older adults.

14.4 Summary and Conclusions

Taking advantage of genetic variations in different mouse strains has yielded much valuable information about structural, functional, and genetic mechanisms that change with age in the cochlea and central auditory system. Characteristics of peripherally induced central auditory plasticity have been elucidated utilizing the C57 strain (rapid hearing loss due to cadherin 23 gene mutation) and the CBA strain (slower ARHL), including rewiring and plastic organizational changes in the brainstem auditory system. Modulation of the peripheral and central biomarkers of presbycusis may be possible with both pharmacological and even acoustic biotherapeutic interventions. Gene expression studies in aging CBA mice yield candidate genes involved in presbycusis that can be examined further in human genetics experiments, including families of apoptotic, antioxidant, neurotransmitter, and immune system genes. Much less is known about human genes involved in presbycusis, with only one gene, *GRM7*, which codes for an important glutamate receptor protein involved in hair cell/auditory nerve synaptic transmission, achieving genome-wide significance and confirmatory replication in multiple human subject cohorts. In closing, we hope that the wealth of knowledge about the genetics of presbycusis gleaned from animal models can now impact on human clinical studies and epidemiological investigations, in terms of experimental design and statistical strategies, more directed health questionnaires, more specific audiometric assessments, and of course tighter comparisons with gene assays. Hopefully, the animal findings will now provide more targeted directions for the human work on candidate genes and pathways for age-related hearing loss.

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