PHARMACEUTICAL INTERVENTIONS FOR HEARING LOSS (PIHL)

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The Genetic and Epigenetic Basis of Noise-Induced Hearing Loss

Royce E Clifford MD, Michael E Hoffer MD, Rick A Rogers PhD

Noise-induced hearing loss can be examined using the model of the junction of environmental effects on the genome and its subsequent expression in phenotype. Individual human susceptibility to noise depends on the type of noise – continuous versus impulse versus explosive, as well as intensity and frequencies, acting in concert with the cellular reaction determined by an individual's DNA (Taggart et al., 2001). Putative genes and their single nucleotide polymorphisms (SNPs) have been studied in humans and in the laboratory; however, the application of animal studies to humans is challenging, due to the varying extent of noise exposure in both industrial and military populations, different SNPs between human and animals, and the need for population studies large enough to demonstrate statistical significance (Annelies Konings, Van Laer, & Van Camp, 2009). Over the last decade, human and laboratory studies have concentrated on candidate genes based on oxidative stress and apoptosis pathways, potassium channel recycling in the inner ear, tip-link genes, and Hsp70 genes (Sliwinska-Kowalska & Pawelczyk, 2013).

The cochlea maintains constant metabolic activity; mechanosensory outer hair cells (OHCs) respond to sound stimuli by adjusting length and breadth, and nerve cells fire a constant baseline stream of signals to the brain. The synthesis of reactive oxidative species (ROS) is a key factor in the maintenance of cellular equilibrium and it is the cell's ability to neutralize ROC species via glutathione, methionine sulfide reductase and other pathways to support cellular metabolism in response to noise exposure. However, when stimulated by excess noise, cochlear cells produce increasing levels of ROS, overwhelming this protective mechanism. ROS that linger in the cell without removal

can cause damage to membranes, DNA, and proteins. Any defect in one of the proteins involved either in neutralizing or in reconstituting the normal glutathione level may decrease the cochlea's ability to respond to this stress, resulting in apoptosis or cell death.

Simultaneously with reaction to noise, the cochlea must maintain its electric potential across two fluid compartments, the scala media and scala vestibulae. Other studies suggest a SNP dysregulation of potassium channels across this fluid barrier may lead to degradation of the potential (Sliwinska-Kowalska & Pawelczyk, 2013).

Epidemiologic genetic studies have implicated SNPs occurring in oxidative pathway genes as well as potassium recycling genes, including glutathione S-transferase (GSTM) isoforms, GSTP, glutathione peroxidase (GPX1), glutathione S-reductase (GSR), superoxide dismutase (SOD), paraoxonase (PON2), catalase (CAT), KCNE1, and others (Carlsson et al., 2005; Fortunato et al., 2004; A Konings et al., 2007; Laer et al., 2006; Lin & Wu, 2009; Rabinowitz et al., 2002). The results may hold promise in directing therapeutic intervention. Lin & Wu (2009) reported that those members of the population who had glutathione S-transferase (GST) T1 and M1 SNPs responded to N-acetylcysteine more consistently than workers exposed to industrial noise with either GST-T1 or GST-M1 SNPs in prevention of temporary threshold shifts. Different regional populations may have diverse SNPs because of global genetic drift, mitigating against drawing general conclusions from a specific population. Thus what may be an important SNP in one part of the world may not be relevant on another continent. In addition, these SNPs have focused on those occurring in exons; whereas SNPs in introns have yet to be addressed. Increasing evidence shows that introns can be involved in control of up regulation, down regulation, enhancement, and directing RNA-replication via epigenetic means.

Research in various animal species has identified genes, proteins, and chromosome areas associated with acoustic susceptibility. This includes oxidative pathways as well as apoptosis genes, genes involved in the initial immune and inflammatory response, and others. Genes crucial to OHC and IHC survival after acoustic trauma include caspase-3, caspase-9, bcl-2, c-Jun N-terminal kinase (JNK), p38 mitogen-activated protein kinases (Cheng, Cunningham, & Rubel, 2005; Minal Patel et al., 2013; Ruan et al., 2007; Yamashita, Minami, Kanzaki, Ogawa, & Miller, 2008), as well as proteins involved in inflammation, i.e., ICAM-1, NF kappaβ, 5 (MCP-5), monocyte chemoattractant protein 1 (MCP-1), MIP-1beta, P-selectin, PECAM-1, IL-6 (Adams, Seed, Lu, Landry, & Xavier, 2009; Seidman et al., 2009; Shi & Nuttall, 2007; Tornabene, Sato, Pham, Billings, & Keithley, 2006; Wakabayashi et al., 2010). Whether these correlate with human SNPs will require population studies.

Another approach to clarification of relevant genes has included quantitative trait locus (QTL) maps for mouse strains resistant to acoustic trauma (Ohlemiller, 2008; Street et al., 2014). QTL mapping identifies stretches of the genome that are involved with protection from noise trauma on different chromosomes in mouse models, and will be helpful in identifying both relevant proteins as well as non-transcribed areas of the

mouse genome. Matching these stretches of chromosome with relevant human areas is possible with bioinformatics methods that are publically accessible.

Clearly SNPs are not be the entire picture, and it is evident that there are numerous epigenetic factors in play as well, including methylation patterns of DNA, histone markers, and RNA regulation both pre-and post-transcription and translation. Changes in expression of enzymes can be controlled by other proteins or methods, i.e., RNA inhibition (RNAi), and thus may not be evident in studies of SNPs. Differential gene expression measurement following acoustic trauma has helped to identify up-regulation and down-regulation of cell systems occurring in response to noise associated with the immune system and inflammation, transcription factors, cytokines, protein synthesis, metabolism, cytoskeletal proteins, calcium balance, oxidative, apoptotic systems, and heat-shock proteins (Cho, Gong, Kanicki, Altschuler, & Lomax, 2004; Gratton et al., 2011; Han et al., 2012; Kirkegaard et al., 2006; Ohlemiller, 2008). These elucidated pathways will be important for studies of therapeutic intervention for prevention of hearing loss as well as treatment. Identification of individuals who have deficits in these pathways can help drive preventive treatment modalities.

In the past decade, it has become clear that protein pathways are not the entire picture. Only 20% of the genome is translated into proteins; on the other hand, greater than 80% of DNA is transcribed into RNA. Untranslated DNA is no longer mistakenly considered "junk DNA," but is transcribed into long non-coding RNA (lncRNA) greater than 200 nucleotides in length, microRNA (miRNA) and small nucleolar RNA (snoRNA) of approximately 20-22 nucleotides in length. These RNAs are involved in expression of genes pre- and post-transcription.

MicroRNAs, non-coding RNAs of 20-22 nucleotides, modulate mRNA level by degradation through the RISC pathway (Patel, 2013). Thus, these provide post-transcriptional/pre-translation control of RNAs, and have been characterized pre- and post-acoustic trauma in the lab (M. Patel & Hu, 2012; Minal Patel et al., 2013). Both up-regulated and down-regulated miRNAs have been described, predominantly associated with cell death and apoptosis.

It is now estimated that there are greater than 60,000 lncRNAs. Those that have been identified sustain histone methylation identical to active promoters of DNA, are expressed at low levels, and are less conserved than protein-coding transcripts. They usually have the genetic structure of 1-2 exons. The study of lnRNAs is in its infancy, but some appear to bring together trans- elements of DNA sequences and recruit epigenetic modifiers, i.e., methylation of histones. Polycomb repressor proteins are complexes that contain 20% expressed lncRNA, and may write and erase chromatin marks, regulate enhancers, and thus regulate the 3D structure of chromosomes with looping and activation of distant promoters (Kornfeld & Brüning, 2014). In the cochlea, Meg3/Gtl2, a maternally-expressed RNA has been characterized important for development (Manji et al., 2006), and others have been associated with tissue homeostasis (lnc-RAP and HI-LNC) and inflammation (Cui et al., 2014).

In conclusion, genes and SNPs involved in NIHL remain a fruitful topic for investigation, the study of epigenetics, i.e., lncRNA, miRNA, is a promising field yet in its infancy. Appropriate tools are being developed and refined to explore the interface of environment and cellular mechanisms. New avenues of research in the areas of characterization of the cochlea's response to stress, and in the field of therapeutic intervention will yield important insight into the mechanism of NIHL.

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HCE Email: sharon.bryant.4.ctr@us.af.mil, Phone: 210-292-4100. COL Mark Packer, Director. Tanisha Hammill, Editor, Email: tanisha.hammill.ctr@us.af.mil, Phone: 210-292-5641