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A Feasibility Protocol to Examine the Use of Genetic Biomarkers for Noise-Induced Hearing Loss in a Sample of Military Personnel

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14. ABSTRACT

Noise-induced hearing loss (NIHL) and age-related hearing impairment are two of the most common conditions demonstrating sensory injury to the auditory nerve, specifically cochlear hair cells damaged in hearing loss (Clifford et al., 2019). NIHL can result from a single exposure to an intense sound (e.g., explosion) or from continuous exposure to noise over a long period of time (e.g., occupational exposure to loud machines). Precision medicine can use genetic biomarkers as indicators of normal biological processes, disease states, and pharmacological responses to therapeutics. Being able to anticipate who might be more susceptible to NIHL using genetic markers could improve military readiness and performance, and reduce service-related disability.

15. SUBJECT TERMS

Noise-induced hearing loss; biomarkers; genetic markers; military; SNPs

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INTRODUCTION

Chronic noise exposure is a side-effect of the industrial age and mechanized warfare. Noise-induced hearing loss (NIHL) and age-related hearing impairment are two of the most common conditions demonstrating sensory injury to the auditory nerve, specifically cochlear hair cells damaged in hearing loss (Clifford et al., 2019). NIHL can result from a single exposure to an intense sound (e.g., explosion) or from continuous exposure to noise over a long period of time (e.g., occupational exposure to loud machines). In military populations, occupational noise hazards can impair readiness and ultimately cause disability. Sheffield et al. (2017) examined the relationship between hearing acuity and operational performance in simulated dismounted combat. They found that impaired hearing caused participants to be less lethal with a higher incidence of mortality. In addition to impacting military operations, hearing loss is significantly associated with depression, isolation, reduced social activity, and cognitive decline (Dawes et al., 2015). Being able to anticipate who might be more susceptible to NIHL could improve military readiness and performance, and reduce service-related disability.

Precision medicine can use genetic biomarkers as indicators of normal biological processes, disease states, and pharmacological responses to therapeutics. The current literature implicates at least 83 genetic loci responsible for hearing loss (Zhang et al., 2019). Many of these genes are involved with the elimination of reactive oxygen and nitrogen species that accumulate in the inner and outer hair cells. Zhou et al. (2014) conducted a meta-analysis of five case control studies of glutathione S-transferase GSTM1 and GSTT1 polymorphisms relative to NIHL. Both genes affect the elimination of reactive species. The meta-analysis showed that persons with GSTM1 null genotype had an increased risk of NIHL compared with those with GSTM1 wild genotype, and that there was no significant difference in the risk of NIHL between persons with GSTT1 null and wild genotypes. Replicated findings in candidate gene studies have included SNP variants of genes encoding antioxidants catalase, superoxide dismutase type 2, paraoxonase 2, and glutathione transferases GSTM1 and GSTT1 (Abreu-Silva et al., 2011; Carlsson et al., 2005; Fortunato et al., 2004; Li et al., 2013; Wang et al., 2014; Yang et al., 2015).

Transcription factors, ion-channels, and integral membrane proteins have also been identified as playing a role in hearing loss, with some genes implicated specifically in NIHL. A SNP identified in the Cadherin 23, which encodes for a component of stereocilia tip links, has been linked to NIHL (Kowalski et al., 2014). A study of Taiwanese soldiers found that the presence of a polymorphism in the WFS1 gene predicted hearing loss in soldiers exposed to gunfire (Yuan et al., 2012). WFS1 codes for a protein called Wolframin, which is located in the endoplasmic reticulum and is thought to help regulate the amount of calcium in cells. The GRHL2 gene (Grainyhead-like Transcription Factor 2) has been implicated in cases of sensorineural hearing loss and contains four polymorphisms that may be related to NIHL (Li et al., 2013). The CAT gene, responsible for making subunits of the enzyme Catalase, also has four polymorphisms associated with risk for NIHL (Konings et al., 2007). The KCNQ4 gene encodes a protein for potassium channels. Three polymorphisms of this gene have been identified as related to NIHL, with one polymorphism being protective (Guo et al., 2018; Van Laer et al., 2006). PCDH15

encodes for integral membrane proteins; one PCDH15 polymorphism has been linked to NIHL (Xu et al., 2017).

While many genetic components have been identified with potential causal links to NIHL, more research is needed to understand if these loci can serve as predictive biomarkers. Here, we describe a research protocol used to isolate genomic DNA (gDNA) from frozen saliva samples originally collected by the Department of Defense Hearing Center of Excellence for a study titled "Hearing Health Education Delivery Using a Precision Preventive Approach" (MRDC IRB # M10690). The original aim of the study was to identify potential genetic biomarkers that might serve as indicators for preventive hearing healthcare. Although the progress of the study was halted prior to its completion, preparatory work performed for the protocol, as reported here, will benefit future efforts to understand how certain genes may play a role in NIHL.

METHODS[1]

This protocol planned to use a subset of data and saliva samples from subjects who participated in the 2018 study titled "Hearing Health Education Delivery Using a Precision Preventive Approach." Participants must have been enrolled in the original study and given permission to store their collected saliva samples and audiogram data for future use.

From those who qualified, audiogram data were analyzed for notch indices and scored on a scale of -1 to +1 (Rabinowitz et al., 2006). Initially, 20 samples that scored close to -1 and +1 were selected for analysis; however, further analysis of the notch indices revealed that most of these samples did not meet the needs of the study. Specifically, negative notch indices were indicative of age-related hearing loss, an unwanted confound. Positive notch indices were suggestive of noise-induced hearing loss, of interest to the study. Audiograms with notch indices close to 0 would indicate normal hearing, also of interest as control. Therefore, 16 additional datasets were selected that had notch values of +1 and 0, rather than +1 and -1.

In the end, 30 frozen saliva aliquot samples were procured from a 59th Medical Wing IRB-approved data repository titled "Hearing Center of Excellence, Hearing Health Education Delivery Using a Precision Preventative Medicine Approach Research Data Repository" (FWH20200072H), which was located at the 59th Medical Wing Research Laboratory. Saliva samples were stored at -80° in QIAGEN's RNAprotect Saliva Reagent. Genomic DNA from the saliva samples were isolated using QIAamp DNA mini kit following the QIAGEN supplementary protocol. Samples were further cleaned and concentrated with Zymo DNA Clean & Concentrator Kit and concentration was determined using fragment analysis.

From the 30 saliva samples processed for gDNA, 20 gDNA samples were selected based on DNA yields of 100 ng or more total gDNA. These 20 samples were sequenced on the NovaSeq 6000 platform using NovaSeq Reagent kit S4. BCL files were transferred to FASTQ files on

[1] The methods and procedures described here were approved in an original study protocol, "An Examination of Genetic Biomarkers of Noise Induced Hearing Loss in a Sample of Military Personnel," approved as by the institutional review board of the U.S. Air Force's 59th Medical Wing (FWH20200075E, March 31, 2020).

DRAGEN and then transferred to external hard drives for storage as VCF files. The resulting files have not yet been analyzed for genetic markers. A VCF was reported for each participant.

ANALYSIS

Analyses were never completed on the resulting sequences due to budgetary and time constraints.

DISCUSSION

The initial objective for this study was to evaluate the validity of genetic biomarkers for NIHL in a sample of military personnel. Second, we wanted to determine the feasibility of similar future work using the full dataset from the 2018 "Hearing Health Education Delivery Using a Precision Preventative Approach" study. While we were unable to address our first objective, we were able to design and implement a method to identify potential NIHL subjects and isolate their gDNA from saliva for sequencing.

Using notch indices method outlined by Rabinowitz et al. (2006), we were able to identify potential subjects for our study. Rabinowitz defined the notch index as the difference between the pure-tone average of thresholds at 2, 3, and 4 kHz and the average of thresholds at 1 and 8 kHz in the same ear. When the audiogram plotted between 1 to 8 kHz is a straight line, the notch index is zero and represents unaffected hearing. If thresholds at 2, 3, and 4 kHz are above the line connecting the 1 and 8 kHz thresholds, as seen in presbycusis, the notch index value would be negative. When the thresholds for 2, 3, and 4 kHz are below the line between 1 and 8 kHz, the notch index is greater than zero and the hearing loss pattern can be considered due to NIHL. This method provided a good objective measure for identifying our two subject populations (i.e., NIHL and control minimal hearing loss). Starting off with two separate populations that had correlating audiograms from an unbiased dataset would have made any identified genetic loci valid candidates for NIHL association.

As with most sequencing studies, the amount and quality of starting material is important. One saliva sample from each subject was collected during the initial "Hearing Health Education Delivery Using a Precision Preventive Approach" study and split into three aliquots for storage. Processing one aliquot per subject did not yield enough gDNA for sequencing, so multiple aliquots from the same subject had to be pooled to get enough DNA to analyze. Because of this, little saliva was left for additional studies. As a biorepository, enough sample and information needed to be collected initially to make the repository useful. In the end, all of the samples were either used or destroyed from the repository, making the effort of setting up the biobank informative but futile.

More research is needed to study the role genes may play in relation to NIHL. If biomarkers and genetic screening can be applied to identify individuals who are predisposed to NIHL, prevention, protection, and intervention strategies can be developed and targeted to preserve readiness and prevent long-term disability in at-risk military members. Identifying genetic markers that may predispose individuals to hearing loss due to noise exposure could help

mitigate the two most prevalent service-connected disabilities of tinnitus and bilateral hearing loss (Veterans Benefits Administration Annual Benefits Report, 2021).

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ABBREVIATIONS AND ACRONYMS

Binary Base Call (BCL)
Defense Health Agency (DHA)
Deoxyribonucleic acid (DNA)
Department of Defense (DoD)
Genomic DNA (gDNA)
Hearing Center of Excellence (HCE)
Institutional Review Board (IRB)
Kilohertz (kHz)
Noise-induced hearing loss (NIHL)
Research and Engineering (R&E)
Ribonucleic acid (RNA)
Single-Nucleotide Polymorphism (SNP)
US Army Medical Research and Development Command (MRDC)
Variant Call File (VCF)

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