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# Review of significant physiological pathways and genes associated or linked with acquired sensorineural hearing loss in humans and mice: A comparative analysis

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# Review of significant physiological pathways and genes associated or linked with acquired sensorineural hearing loss in humans and mice: A comparative analysis

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**Abstract**

There has been increased studies in genetic polymorphisms for acquired sensorineural hearing loss (ASNHL). Many of these studies identify genes related to single cochlear insults such as age-related, noise-induced, or ototoxic hearing loss. These insults have overlapping molecular mechanisms. In a recent study, we used a cluster analysis to analyze genes related to ASNHL for all three cochlear insults to identify biological processes that generalize to ASNHL. In this study, we compared genes and biological processes highlighted in the previous study with the genes and biological processes highlighted in systematic reviews of ASNHL caused by individual insults to determine which new genes and processes were identified in our recent analysis. Biological processes found in our recent studies systematic review, but not other systematic reviews include folic acid metabolism and blood vessel diameter regulation. Identifying these new biological processes has demonstrated that a broader systemic review and cluster analysis of genes related to ASNHL reviled pathways otherwise missed by more focused reviews of ASHNL caused by individual cochlear insults. The results of our recent analysis also confirmed the association of oxidative stress, potassium recycling and many other biological processes identified in previous reviews.

## Introduction

A systematic review was conducted to collect original research relating specific genes with age-related hearing loss (ARHL), noise-induced hearing loss (NIHL), or ototoxicity. All three cochlear insults are a part of acquired sensorineural hearing loss (ASNHL). Fifty-six genes were found to be related to ASNHL in humans or mice (Appendix 1). Ontological enrichment analysis was run to identify the ontological classes overrepresented among these sets of genes. This shows these genes are involved in many different biological processes (Appendix 2). The purposes of the systematic review and ontological enrichment analysis were to identify significant associations with ASNHL and indicate the biological processes in physiological pathways. It is the first study to systematically include all three acquired cochlear insults, age, noise, and toxin-induced hearing loss, and to provide a holistic analysis of the associated genes. The aim of this paper is to compare our findings of genes associated with ASNHL to the genes found in other reviews. This paper aims to summarize important genetic polymorphisms associated with ASNHL that appeared in multiple reviews and describe their role in biological pathways. A total of seven articles were found to conduct this comparative analysis. Five articles were found with the purpose of reviewing human or animal research to discuss genes associated with either age or noise-induced hearing loss (Konings et al., 2009; Sliwinska-Kowalska et al., 2012; Ciorba et al., 2015; Bowl et al., 2019; Miao et al., 2019). One article was found with the purpose of organizing genomic and epigenetic data from NIHL studies into cellular pathways (Clifford et al., 2016). The final article was found with the purpose of reviewing epidemiological studies that have shown linked deficiency in nutrition and homocysteine metabolism to sensorineural hearing loss (SNHL) (Partearroyo et al., 2017). We review the relationship between hearing loss and folic acid because it was the most significant pathway in our ontological analysis (as highlighted in Appendix 2). None of the reviews had identified any genes associated specifically with toxin-induced hearing loss focusing solely on either noise-induced hearing loss or age-related hearing loss.

The following genes were related to ASNHL in at least 2 research studies, CDH23**,** GSTT1**,** HSPA1A1**,** NAT2**,** NOX3**,** NRF2**,** SOD1**,** SOD2**,** SLC5A7**,** GSTM1**,** GRM7**,** FSCN2**,** and AQP4. Only six of these genes were mentioned in other systematic reviews. The genes discussed in this paper were related to ASNHL and mentioned in at least two past review articles (Table 1). Only one gene, MTHFR, is listed under one review. Genes PCDH15 and MYH14 were mentioned in two reviews, but these genes were not found in our systematic search.

**Table 1.**

| Genes | Bowl et al. 2019 (ARHL) | Ciorba et al. 2015 (ARHL) | Konings et al. 2009 (NIHL) | Sliwinksa-Kowalska et al. 2012 (NIHL) | Miao et al. 2019 (NIHL) | Partearryo et al. 2017 (SNHL) |
|---|---|---|---|---|---|---|
| **SOD1** | | | | | | |
| CAT | | | | | | |
| **SOD2** | | | | | | |
| **CDH23** | | | | | | |
| **GSTM1** | | | | | | |
| **HSPA1A** | | | | | | |
| **GRM7** | | | | | | |
| KCNE1 | | | | | | |
| PON2 | | | | | | |
| MTHFR | | | | | | |
| KCNQ4 | | | | | | |
| HSPA1L | | | | | | |
| GRHL2 | | | | | | |
| PDH15* | | | | | | |
| MYH14* | | | | | | |

Table 1 shows genes significantly associated with hearing loss and mentioned in multiple reviews. The **bolded** genes are significant to our systematic review. The genes denoted with an asterisk* were not found in our systematic search.

## Physiological Pathways and Genes Related to ASNHL

There are many physiological pathways involved in the hearing process. Genes in these pathways have many roles to regulate the process. Some gene polymorphisms or mutations may increase or decrease susceptibility to ASNHL. A few of the significant biological process groups and ontological classes overrepresented by our genes related to ASNHL that will be discussed are response to folic acid, response to hydrogen peroxide, and response to oxidative stress. Similar to our ontological class findings, Clifford et al. 2016 identified significant pathways related to cellular stress response, heat stress response, and detoxification of reactive oxygen species (ROS). Other reviews also found genes in oxidative stress, potassium ion (K+) recycling pathway, and heat stress response. Monogenic deafness genes and genes encoding adhesion molecules are also significant biological processes related to ASNHL. The following sections will describe these different pathways and the genes involved along with their significant association with age-related or noise-induced hearing loss.

### I. Metabolism, Nutrition, and Genes associated with Sensorineural Hearing Loss

The current study found a significant overrepresentation of genes involved in response to folic acid. Based on our ontological enrichment analysis, out of 1018 genes that contribute to the biological processes of response to folic acid, 20 genes were also related to ASNHL. Six of those genes are discussed in this paper. The role of CAT, KCNQ1, and SOD1 and their association in ASNHL were described above. The genes GJB2, MTHFR, and TYMS were found to have a direct role in response to folic acid. The response to folic acid has been an overlooked pathway in previous reviews as we are the first to highlight the pathway and its genes related to ASNHL.

Different epidemiological studies have shown correlations among homocysteine metabolism and folic acid deficiencies and SNHL (Cohen-Salmon et al., 2007; Kundu et al., 2012). Knockdown of connexin 30 (Cx30; also known as GJB6) gene, a gap junction protein, is associated with changes in cochlear homocysteine (Hcy) metabolism. As seen in Cx30-/- mice, this causes the stria vascularis (SV) fail to produce an endocochlear potential (EP), and results in

severe congenital hearing impairment. The mice had significant down-regulation of betaine homocysteine S-methyltransferase (Bhmt) in the SV which resulted in increased Hcy, a known factor of endothelial dysfunction (Cohen-Salmon et al., 2007). Randomized trials showed a decrease in total plasma homocysteine (tHcy) levels by dietary folate supplementation (Jacquesetal.,1999), and an intervention trial by Durga et al. (2007) showed FA supplementation slowed ARHL in a population from a country without folic acid enrichment of food. Additional animal model studies have been conducted to understand the effect of folic acid in the diet. Cbs+/− heterozygous mice showed moderate sensorineural hearing loss (SNHL), increased levels of Hcy in the SV and the spiral ligament, oxidative stress and decreased vessel density. These effects were avoided when folic acid (FA) was added to the drinking water (Kundu et al., 2012). Martínez-Vega et al., 2015 found the mouse strain C57BL/6J mice, susceptible to SNHL, showed increased hearing thresholds after 2 months on a FD (folate deficiency) diet. This reduced serum folate concentrations and increased tHcy. These results show the relationship between HHcy and premature SNHL involves cellular degeneration, impairment of cochlear Hcy metabolism and associated oxidative stress. The aforementioned results were corroborated in research on the long-term effects of FD on a strain found in mice. The mouse strain had a prolonged SNHL onset, a deficiency that resulted in premature SNHL (Martínez-Vega et al., 2016). Further research needs to be conducted on the effects of the folic acid diet in humans and its correlations with homocysteine metabolism and SNHL.

Reduced FA concentrations found in age-related hearing loss (ARHL) and sudden SNHL, correlate with reduced vitamin B12 (Houston et al., 1999; Lasisi et al., 2010; Karli et al., 2013) or increased tHcy levels (Cadoni et al.,2004). Hcy concentrations show variations in its metabolism, which include the methionine and folate cycles, as well as the transsulfuration pathway. However, there is no consistent association between mutations in genes of these pathways and SNHL as seen in the analysis of the putative relationship between the methylenetetrahydrofolate reductase (MTHFR) C677T mutation and ARHL (Durga et al., 2006; Uchida et al., 2011; Fusconi et al.,

2012). A study on ARHL found the relationship between serum folate and hearing thresholds dependent on MTHFR 677 genotype and independent of homocysteine. The results showed high serum folate concentrations were associated with lower hearing thresholds in MTHFR 677TT and with higher hearing thresholds in MTHFR CC. When folate was below the median, MTHFR 677TT had similar hearing levels as subjects with a C allele, but when folate was above the median the MTHFR 677TT genotype may have offered protection against hearing loss (Durga et al., 2006). The same protective effect of the MTHFR 677T allele in ARHL was found by Uchida et al. 2011, but only when the subjects had the AA genotype for MTR A2756G. The same study showed the association of the MTHFR 677T allele ARHL was independent of folate and Hcy level, while plasma tHcy was independently associated with ARHL. Contradicting these studies, Fusconi et al. 2012 found a statically significant correlation between the MTHFR C677T mutation and hyperhomocysteinemia (HHcy) in sudden SNHL. A recent pilot study presented a significant association of the genes MTHFR 677TT and TYMS with ARHL (Manche et al. 2018). As seen by the multitude of studies, MTHFR has been associated with SNHL. Studies have yet to agree on the gene's relationship in homocysteine metabolism and serum folate levels. Therefore, further research is needed to support its association with SNHL, whether that be protective or at risk.

## II. Oxidative Stress

Oxidative stress has been a widely studied physiological response and is well established in the cochlea as a mechanism for ASNHL. Our analysis has confirmed these findings. All seven reviews mentioned oxidative stress in the development of age-related and noise-induced hearing loss. Oxidative stress occurs when cells experience excessive amounts of oxidants or decreased levels of antioxidants in different parts of the body. This biological phenomenon has led many to study its effects in hearing loss (Henderson et al., 2006; Darrat et al., 2007). Noise exposure has shown an initial increase in cochlear blood flow followed by an abrupt decrease in cochlear circulation seen by an aggregation of RBCs, capillary vasoconstriction, and stasis (Quirk & Seidman, 1995). Decreased cochlear blood flow from noise exposure alters cellular redox states

and causes formation of free radicals (Evans & Halliwell, 1999). Overexpression of free radicals can damage cellular DNA, proteins, lipids, and unregulated apoptotic pathways, causing cell death and irreversible damage to the cochlea (Campbell et al., 2003). Impaired oxidative defense mechanisms, such as depletion of enzymatic and non-enzymatic antioxidants can also lead to oxidative stress (Figure 1) (Hovatta et al., 2010). Genes involved in the oxidative stress response include CAT, GSTM1, PON2, SOD2, and SOD1. These genes were associated with NIHL in three reviews (Konings et al. 2009; Sliwinska-Kowalska et al., 2012; and Miao et al., 2019).

**Figure 1.**

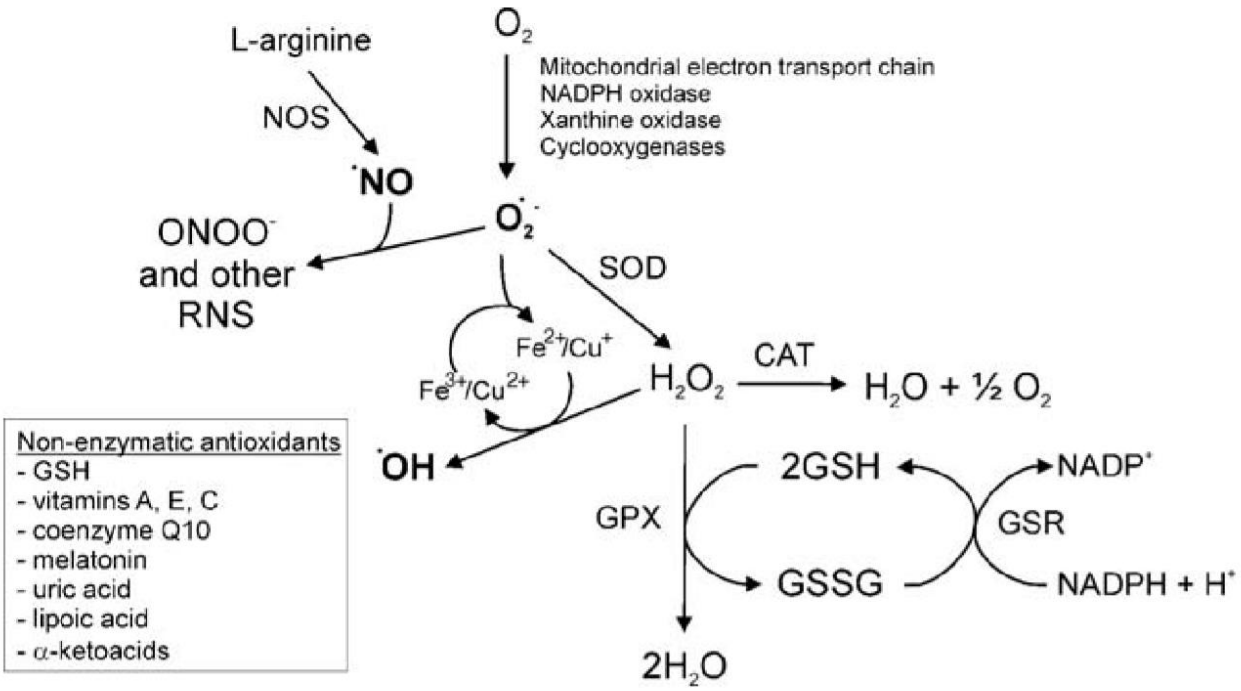


Figure 1 shows the major biochemical pathways of free radical production, and enzymatic and non-enzymatic antioxidative defenses. Abbreviations: CAT: catalase; GPX: glutathione peroxidase; GSSG/GSH: oxidized/reduced glutathione; GSR: glutathione reductase; NADP+/NADPH: oxidized/reduced nicotinamide adenine dinucleotide phosphate; NOS: nitric oxide synthase; RNS: reactive nitrogen species; SOD: superoxide dismutase (Hovatta et al., 2010).

An animal study has shown that catalase (CAT) increases after noise exposure (Jacono et al., 1998). Catalase (hydrogen peroxide oxidoreductase), a heme-containing protein, is an enzyme involved in the breakdown hydrogen peroxide ($H_2O_2$) (Chance et al., 1979). In the inner ear, the organ of Corti has higher levels of catalase than the stria vascularis (Jacono et al., 1998).

Konings et al. 2007 analyzed audiometric data from 1261 Swedish and 4500 Polish noise-exposed laborers. DNA samples from the most susceptible and the most resistant individuals were collected. It was observed that most SNPs showed a significant association to NIHL when exposed to high levels of noise (>92 dB). This indicates that polymorphisms in CAT have a greater effect when carriers are exposed to higher levels of noise. Therefore, noise exposure levels must be considered to determine the effect of CAT polymorphisms. Although the results of the study revealed that catalase is a NIHL susceptibility gene, more research needs to be conducted in other non-European populations.

Glutathione S-transferases (GST) are an important family of phase II drug-metabolizing enzymes that catalyze the conjugation of a large variety of endogenous and exogenous compounds with reduced glutathione. Glutathione S-transferases plays an important role in the antioxidant protection of the cochlea. Genetic polymorphisms of human cytosolic GST genes lead to altered GST activity, which contributes to interindividual differences in the response to xenobiotics (Hayes et al., 2005). Rabinowitz et al. (2002) analyzed GSTM1 deletion polymorphisms in 58 noise-exposed workers. The study showed that carriers of the GSTM1 gene were protected from NIHL. However, both Konings et al., 2009 and Sliwinska-Kowalska et al. 2013 acknowledge that the small sample size causes the study to lack power and makes this a spurious association. This shows that more research needs to be done in larger populations to identify a true association between GSTM1 and NIHL.

Superoxide is generated in the cochlea after acoustic overstimulation (Henderson et al., 2006). Therefore, regulating superoxide levels is important for cochlear degeneration caused by ROS. The SOD enzyme converts superoxide radicals to hydrogen peroxide. Noise exposure is seen to produce high levels of SOD1 (superoxide dismutase 1) enzymes in the cochlea and may have direct functional implications. Lack of SOD1 enzymes has led to an increase acoustic trauma related hearing loss. An animal model study using mice showed those without SOD1 were more susceptible to NIHL than their wild-type littermates (Ohlemiller et al. 1999). In a human study, Liu

et al. 2010 found rs2070424 and rs10432782 in SOD1 are associated with NIHL in Chinese workers. These SOD1 SNPs showed statistically significant differences between the noise-sensitive workers and the noise-resistant workers in genotypic and allelic distributions. The AA genotype of rs2070424 offered protection against NIHL and the GG genotype of rs10432782 was associated with a higher risk suggesting that polymorphisms of SOD1 may lead to NIHL in the Chinese population. The significance of SOD1 in NIHL needs to be further studied in another diverse human population.

Miao et al. 2019 claimed that the oxidative stress gene polymorphisms associated with NIHL, SOD2 rs2855116 and PON2 rs12026 and rs7785846 were the most important SNPs. Fortunato et al. 2004 analyzed polymorphisms in the antioxidant genes paraoxonase 2 (PON2) and superoxide dismutase 2 (SOD2) in 94 European male workers exposed to noise and revealed a significant association with NIHL. The study results show carriers of PON2 and SOD2 polymorphisms may be susceptible to NIHL. Similar results for SOD2 association were replicated by Chang et al. (2009) which analyzed SOD2 polymorphism rs2855116 in 200 Taiwanese factory workers. The study found individuals with T/G genotype of SOD2 polymorphism were more susceptible to noise. Two independent studies have supported the role of SOD2 in NIHL.

### III. Potassium Ion Recycling

The endolymph fluid that fills the scala media contains a high concentration of K+. Potassium carries the charge for sensory transduction. Deflection of the hair bundle causes K+ from the endolymph to enter the hair cells through mechanosensitive K+ channels. It leaves the cells through basolateral K+ channels and recycles back to the endolymph following two routes via the spiral limbus or the stria vascularis (Couloigner et al. 2006; Wangemann 2002). These K+ recycling pathways are essential for the hearing process. This is supported by mutations in genes involved in K+ recycling (GJB6, KCNE1, and KCNQ4), that cause hearing loss (Grifa et al. 1999; Kubisch et al. 1999; Schulze-Bahr et al. 1997). This K+ recycling pathway and the genes KCNE1 and KCNQ4 were included in three NIHL genetic association reviews (Konings et al., 2009;

Sliwinksa-Kowalska et al., 2012; Miao et al., 2019). Van Laer et al. 2006 genotyped and analyzed SNPs in genes known to be involved in K+ recycling for their association with NIHL in a Swedish population. The results showed three SNPs in KCNE1 and 1 SNP in KCNQ4 may be significantly associated with NIHL. These results were replicated in Polish noise-exposed workers in the same study. The significant polymorphisms KCNE1 rs2070358 and KCNQ4 Q455H were the same as those reported in the Swedish sample set. However, the direction of the genetic trend for KCNQ4 in the Swedish population was opposite in the Polish sample. Possible explanations are differences in allele frequencies or linkage disequilibrium (LD) patterns in the both populations, slightly different selection procedures applied in both studies, the influence of various environmental factors, or a possible false-positive association with NIHL (Pawelczyk et al. 2009). Although there are differences presented, the replicated results in two independent sample sets shows favor towards an association of KCNE1 and KCNQ4 with NIHL.

## IV. Heat Shock Protein

Heat shock proteins (HSPs) are well-preserved proteins expressed in cells under both physiological and pathological conditions. They assist in the synthesis, folding, assembly, and intracellular transport of many proteins. HSPs are produced in the cochlea after acoustic overstimulation (Lim et al. 1993; Samson et al. 2007). Yang et al. (2006) genotyped three SNPs in three HSP70 genes (rs1043618, rs1061581 and rs2227956) in 194 noise-exposed Chinese autoworkers. There was no detection of significant associations with NIHL in single SNP, but there was a significant association between haplotypes GGC and GGT and NIHL. In a similar study, Konings et al. 2009a genotyped the same three polymorphisms in HSP70 genes in 206 Swedish and 238 Polish samples. In the Swedish sample, all three SNPs were significantly associated with NIHL while only rs2227956 resulted in a significant P-value in the Polish sample set. Haplotypes were different when compared to the Chinese study and may be due to the ethnic differences between the sample sets. This study provided more evidence for HSP70 genes susceptibility to

NIHL by replicated the association of HSP70 genes with NIHL in a second and third independent noise-exposed sample set.

## V. Cadherin Protein & Monogenic Deafness Genes

The cadherin 23 (Cdh23) gene encodes a component of the stereocilial tip-link required for gating the mechanoelectrical transducer channel (Siemens et al. 2004; Kazmierczak et al. 2007). Mutations to Cdh23 can lead to hearing loss. Cdh23 is the only gene linked with susceptibility to NIHL in waltzer mice (Holme et al., 2004). The homozygosity at $Cdh23^{753A}$ is a determining factor of age-related hearing loss (ARHL) in mice (Noben-Trauth et al., 2003). A study group of 627 Polish workers exposed to occupational noise yielded a significant association for Cdh23 SNP rs3752752 and NIHL. The effect was observed in young subjects (<35 years old) and those exposed to impulse noise. There was a higher frequency of the CC genotype among noise-susceptible subjects, while subjects resistant to noise had a higher frequency of the CT genotype. These results show that varying CDH23 genotypes alter susceptibility to NIHL in humans. However, the differences seen were not only related to susceptibility to noise, but also age-related cochlear changes, meaning the results should be interpreted with caution (Kowalski et al. 2014). More research needs to be conducted to understand whether CDH23 is truly associated with susceptibility to NIHL.

PCDH15, part of the cadherin superfamily, encodes a protein that mediates calcium-dependent cell-cell adhesion and essential in the preservation of normal retinal and cochlear function. Gene mutations are associated with both non-syndromic (DFNB23, Ahmed et al.,2003) and syndromic hearing loss (Usher syndrome type 1F, USH1F, Alagramam et al., 2001; Ahmed et al., 2001). MYH14, a member of the myosin superfamily, encodes actin-dependent motor proteins with diverse functions including regulation of cytokinesis, cell motility and cell polarity. Gene mutations result in the autosomal dominant hearing impairment DFNA4 (Donaudy et al., 2004; Chen et al., 1995). Konings et al. 2009b analyzed 644 SNPs in 53 candidate genes a Swedish set and a Polish set. The results showed both PCDH15 rs7095441 and MYH14

rs588035 replicated in two independent sample sets. The study suggests PCDH15 and MYH14 genes may be susceptible to NIHL. These genes were not found in our systematic search but their significant association to NIHL was mentioned in two reviews (Sliwinska-Kowalska et al., 2012; Miao et al., 2019).

GRHL2 (grainyhead like 2 gene) is a transcription factor that expressed in different epithelial tissues (Wilanowski et al., 2002). In the cochlea, GHRL2 is expressed in cochlear duct cells, mostly seen in embryonic development and less in early postnatal stages (Peters et al., 2002). GRHL2 SNP rs10955255 was found to be significant in an association study on 2418 age-related hearing impairment (ARHL) European samples. The candidate gene study analyzed 768 SNPs selected in 70 genes. The candidate gene approach showed that GRHL2 is an ARHI susceptibility gene, with highly significant associations in the complete sample set and replication in two independent populations (Van Laer et al. 2008).

Bowl et al. 2019 stated that GRM7 is the strongest gene associated with ARHI because it has been replicated in several independent cohorts (Friedman et al. 2009; Newman et al. 2012). GRM7 (metabotropic glutamate receptor type 7) is a G-coupled receptor activated by L-glutamate which reduces the release of the neurotransmitter glutamate. A study conducted by Friedman et al. 2009 suggested that GRM7 variants may cause ARHI through excitotoxicity. The study used 846 cases and 846 controls selected from six European countries to perform the first whole-genome association study for ARHI. Genotyping and analysis were conducted with a European and Finish sample group. Subsequently, the top-ranked SNPs were genotyped in an independent European replication sample. The results identified a highly significant and replicated SNP located in GRM7. This GWAS shows that variation in GRM7 may be associated with ARHI through a susceptibility to glutamate excitotoxicity (Friedman et al. 2009). A study of a European-American population from Rochester was the first to investigate the genetic associations with measures of speech detection which supported GRM7's role in ARHI. Results showed GRM7 is significantly

associated with pure tone threshold (individual thresholds across the ears) and speech reception threshold. (Newman et al. 2012).

**Conclusion**

Many genes have been associated with acquired sensorineural hearing loss. The different biological pathways these genes are involved in may have significant impacts on the hearing process. This paper reviewed multiple studies that have found genes significantly associated with age-related hearing loss or noise-induced hearing loss. Previous systematic reviews have chosen to collect data on only a single cochlear insult. No review was found to include genes associated with ototoxicity. This paper is the first to collect research data and compile results related to hearing loss in all three cochlear insults including age-related, noise-induced, and toxin-induced. This provided for a holistic analysis of genes associated or linked with ASNHL and their ontological groups. The genes that were found underwent ontological enrichment analysis to demonstrate the important biological processes regulated by these genes. Many significant biological processes found were also discussed in previous reviews.

The genes found significant in our systemic review were CDH23**,** GSTT1**,** HSPA1A1**,** NAT2**,** NOX3**,** NRF2**,** SOD1**,** SOD2**,** SLC5A7**,** GSTM1**,** GRM7**,** FSCN2**,** and AQP4, due to significant association or linkage in multiple studies. In this paper, genes found significant in other reviews were KCNE1, PON2, MTHFR, KCNQ4, HSPA1L, GRHL2, PDH15, and MYH14. Of these additional genes, only PDH15, and MYH14 were not found in our systematic search. The significant pathways discussed in this paper included response to oxidative stress, potassium recycling, heat shock proteins, cadherin proteins, monogenic deafness genes, and response to folic acid. The biological processes involved in folic acid response was highlighted and described in detail for its highly significant value in our ontological enrichment analysis. Further research needs to be conducted on the molecular mechanisms of folic acid in the cochlea and the genes responsible for its response. Also necessary is further research on the various genetic

polymorphisms found significantly associated with ASNHL in additional populations to ensure there truly is an association.

**Appendix 1.** Full list of genes found to be associated with ASNHL

| Human Gene | Variant |
|---|---|
| **SOD1** | rs2070424 (protection) |
| | rs10432782 |
| | HT TATG (protection) |
| | HT CATG |
| | HT TGGA |
| | Whole Gene |
| CAT | rs564250 |
| | rs1001179 |
| | rs494024 |
| | rs12273124 |
| | rs475043 |
| **NRF2** | n/a |
| | rs6721961 |
| | n/a |
| SLC12A2* | n/a |
| TMC1 | n/a |
| TRIOBP | rs58389158 |
| WFS1 | rs62283056 |
| **SOD2** | rs4880 |
| | rs2855116 |
| COL1A1* | n/a |
| GSTM3 | *B allele |
| EYA4 | rs9493627 |
| KCNQ1 | rs163171 |
| **CDH23** | n/a |
| Bak1 | n/a (protection) |

| | |
|---|---|
| **GSTM1** | Null |
| **HSPA1A** | **rs1043618** |
| | rs1061581 |
| CYP1A1 | 2453 C>A |
| | 2455 A>G |
| GJB2 | n/a |
| **GRM7** | **HT 6 (rs11928865/rs9877154)** |
| KCNE1 | rs2070358 |
| ATP2B2* | n/a |
| COCH* | n/a |
| MYO1A* | n/a |
| UCP2 | (−866) G>A |
| PON2 | rs12026 |
| | rs7785846 |
| APE1 | -656 TT |
| OGG1 | Ser326Cys |
| MTHFR | rs1801133 |
| TYMS | rs16430 |
| **NAT2** | **NAT2*6A** |
| NR3C1* | n/a |
| ADGRL3* | n/a |
| PTPRM* | n/a |
| **FSCN2** | n/a |
| KCNQ4 | p.H455Q |
| **NOX3** | **rs33652818** |
| NRTN* | n/a |
| SLC25a23* | n/a |

| | |
|---|---|
| **SLC5A7*** | n/a |
| XIAP | n/a |
| IQGAP2 | rs457717 |
| ILDR1 | rs2877561 |
| ISG20 | rs4932196 (Upstream) |
| **GSTT1** | Null |
| XPC | rs2228001 |
| NCL | rs7598759 |
| SLC16A5 | rs4788863 (protection) |
| HSPA1L | rs2227956 |
| COMT | rs9332377 |
| ZNF22* | n/a |
| **AQP4*** | n/a |
| LOX* | n/a |
| GJA1* | n/a |
| BSND* | n/a |
| TFB1M | n/a |
| GRHL2 | rs10955255 |
| | rs2127034 |
| | rs13263539 |
| | rs606338 |
| | rs1981361 |
| | rs17398001 |
| | rs17399841 |
| | rs7827945 |

The genes in bold were deemed significant to our study for appearing in multiple research studies.

**Appendix 2.** Biological process groups and ontological classes overrepresented in a list of genes related to ASNHL with a false discover rate less than 0.01. The raw p-values, number of genes associated with ASNHL in each ontological class, and the total number of genes from each ontological class among the genome are listed.

| Gene Ontology Biological Process Group | Ontological class | p-Value | Genes related to ASNHL | Total Genes |
|---|---|---|---|---|
| Sensory perception of sound | Sensory perception of sound | 1.33E-18 | 15 | 156 |
| **Response to folic acid** | **Response to drug** | **6.17E-12** | **20** | **1018** |
| Cellular detoxification of nitrogen compound | Response to chemical | 5.10E-11 | 37 | 4496 |
| Aging | Aging | 7.61E-10 | 11 | 282 |
| Auditory receptor cell development | Inner Ear Development | 9.65E-09 | 9 | 201 |
| Response to hydrogen peroxide | Response to oxidative stress | 2.04E-08 | 11 | 391 |
| Cardiac muscle cell action potential involved in contraction | Regulation of biological quality | 9.42E-07 | 29 | 4106 |
| Epididymis development | Developmental Process | 9.92E-07 | 36 | 5901 |
| Response to hyperoxia | Response to abiotic stimulus | 9.95E-07 | 15 | 1161 |
| Xenobiotic catabolic process | Response to xenobiotic stimulus | 2.81E-06 | 8 | 297 |
| Export across plasma membrane | Transmembrane transport | 3.98E-06 | 15 | 1300 |
| Response to copper ion | Response to inorganic substance | 4.01E-06 | 10 | 541 |
| Response to cAMP | Response to organophosphorus | 4.40E-06 | 6 | 141 |
| Cellular response to oxidative stress | Cellular response to oxidative stress | 7.08E-06 | 7 | 238 |
| Cellular homeostasis | Cellular homeostasis | 1.01E-05 | 12 | 895 |
| Hydrogen peroxide biosynthetic process | Reactive oxygen species metabolic process | 2.43E-05 | 5 | 113 |
| Inorganic cation transmembrane transport | Ion Transport | 2.59E-05 | 14 | 1335 |
| Glutathione metabolic process | Glutathione metabolic process | 3.02E-05 | 4 | 57 |
| Positive regulation of blood vessel diameter | Positive regulation of blood vessel diameter | 3.02E-05 | 4 | 57 |
| Sensory organ morphogenesis | Anatomical structure morphogenesis | 3.48E-05 | 18 | 2177 |

The **response to folic acid** is bolded to represent its significance to this paper.

References

Bowl, M.R. & Dawson, S.J. (2019). Age-related hearing loss. *Cold Spring Harb Perspect Med* 2019;9:a033217. doi: 10.1101/cshperspect.a033217.

Cadoni, G., Agostino, S., Scipione, S., and Galli, J. (2004). Low serum folate levels: a risk factor for sudden sensorineural hearing loss? *Acta Otolaryngol. 124*, 608–611. doi:10.1080/00016480410016216

Chance, B., Sies, H. & Boveris, A. (1979). Hydroperoxide metabolism in mammalian organs. *Physiol. Rev., 59,* 527–605.

Chang, N.C., Ho, C.K., Wu, M.T., Yu, M.L., & Ho, K.Y. (2009). Effect of manganese-superoxide dismutase genetic polymorphisms IVS3-23T/G on noise susceptibility in Taiwan. *American Journal of Otolaryngology—Head and Neck Medicine and Surgery 30*(2009); 396-400. doi:10.1016/j/amjoto.2008.08.001

Ciorba, A., Hatzopoulos, S., Bianchini, C., Aimoni, C., Skarzynski, H., & Skarzynski, P.H. (2015). Genetics of presbycusis and presbystasis. *International Journal of Immunopathology and Pharmacology 28*(1): 29-35. DOI: 10.1177/0394632015570819.

Clifford, R.E., Hoffer, M., & Rogers, R. (2016). The genomic basis of noise-induced hearing loss: a literature review organized by cellular pathways. *Otology & Neurotology 37*(8): e309-e316. DOI: 10.1097/MAO.0000000000001073.

Cohen-Salmon, M., Regnault, B., Cayet, N., Caille, D., Demuth, K., Hardelin, J.P., et al. (2007). Connexin30 deficiency causes instrastrial fluid-blood barrier disruption within the cochlear stria vascularis. *Proc. Natl. Acad. Sci. USA 104,* 6229–6234. doi:10.1073/pnas.0605108104

Couloigner, V., Sterkers, O., & Ferrary, E. (2006). What's new in ion transports in the cochlea? *Pflugers Arch, 453,* 11–22.

Darrat, I., Ahmad, N., Seidman, K., & Seidman, M.D. (2007). Auditory research involving antioxidants. *Curr. Opin. Otolaryngol. Head Neck Surg. 2007*, 15, 358-363.

Durga, J. Anteunis, L.J.C., Schouten, E.G., Bots, M.L., Kok, F.J., & Verhoef, P. (2006). Association of folate with hearing is dependent on the 5,10-methylenetetrahydrofolate reductase 677C -> T mutation. *Neurobiology of Aging 27*, 2006, 482-489.

Durga, J., Verhoef, P., Anteunis,L.J. ,Schouten,E., & Kok, F.J. (2007). Effects of folic acid supplementation on hearing in older adults: a randomized, controlled trial. *Ann. Intern. Med. 146,* 1–9. doi: 10.7326/0003-4819-146-1-20070102000003

Friedman, R.A., Van Laer, L., Huentelman, M.J., Sheth, S.S., Van Eyken, E., Corneveaux, J.J., Tembe, W.D., Halperin, R.F., Thorburn, A.Q., et al. (2009). *Human Molecular Genetics, 2009, 18*(4); 785–796. doi:10.1093/hmg/ddn402

Fusconi, M., Chistolini, A., de Virgilio, A., Greco, A., Massaro, F., Turchetta, R. Benincasa, A.T., Tombolini, M., & de Vincentiis, M. (2012). Sudden sensorineural hearing loss: A vascular cause? Analysis of prothrombotic risk factors in head and neck. *International Journal of Audiology,* 51(11), 800-805, DOI: 10.3109/14992027.2012.705904

Grifa, A., Wagner, C. A., D'Ambrosio, L., et al. (1999). Mutations in GJB6 cause nonsyndromic autosomal dominant deafness at DFNA3 locus. *Nat Genet, 23*, 16–18.

Hayes, J.D., Flanagan, J.U., & Jowsey, I.R. (2005). Glutathione transferases. *Annu Rev Pharmacol Toxicol. 2005;* 45: 51–88.

Henderson, D., Bielefeld, E.C., Harris, K.C., Hu, B.H. (2006). The role of oxidative stress in noise-induced hearing loss. *Ear Hear. 2006;* 27: 1–19.

Holme, R.H., & Steel, K.P. (2004). Progressive hearing loss and increased susceptibility to noise-induced hearing loss in mice carrying a Cdh23 but not a Myo7a mutation. *JARO 2004*; 5: 66-79.

Houston, D. K., Johnson, M. A., Nozza, R. J., Gunter, E. W., Shea, K. J., Cutler, G.M., et al. (1999). Age-related hearing loss, vitamin B-12, and folate in elderly women. *Am. J. Clin. Nutr. 69,* 564–571.

Hovattaa, I., Juhilaa, J., & Donner, J. (2010). Oxidative stress in anxiety and comorbid disorders. *Neuroscience Research 68*, 2010, 261-275. doi:10.1016/j.neures.2010.08.007

Jacono, A.A., Hu, B., Kopke, R.D., Henderson, D., Van De Water, T.R. & Steinman, H.M. (1998). Changes in cochlear antioxidant enzyme activity after sound conditioning and noise exposure in the chinchilla. *Hear. Res., 117*, 31–38.

Karli, R., Gül, A., and U˘gur, B. (2013). Effect of vitamin B12 deficiency on otoacoustic emissions. *Acta Otorhinolaryngol. Ital. 33*, 243–247.

Kazmierczak, P., Sakaguchi, H., Tokita, J., Wilson-Kubalek, E.M., Milligan, R.A., Muller, U., & Kachar, B. (2007). Cadherin 23 and protocadherin 15 interact to form tip-link filaments in sensory hair cells. *Nature 449:* 87–91.

Konings, A., Van Laer, L., & Van Camp, G. (2009). Genetic studies on noise-induced hearing loss: a review. *Ear & Hearing 30*(2);151–159.

Konings, A., Van Laer, L., Michel, S., Pawelczyk, M., Carlsson, P.I., Bondeson, M.L., Rajkowsk, E., Dudarewicz, A, Vandevelde, A., et al. (2009a). Variations in HSP70 genes associated with noise-induced hearing loss in two independent populations. *European Journal of Human Genetics (2009)17*, 329 – 335.

Konings, A., Van Laer, L., Pawelczyk, M., Carlsson, P.I., Bondeson, M.L., Rajkowska, E., Dudarewicz, A., et. al. (2007). Association between variations in CAT and noise-induced hearing loss in two independent noise-exposed populations. *Human Molecular Genetics, 2007,16*(15); 1872–1883. doi:10.1093/hmg/ddm135

Konings, A., Van Laer, L., Wiktorek-Smagur, A., Rajkowska, E., Pawelczyk, M., Carlsson, P.I., Bondeson, M.L., Dudarewicz, A, Vandevelde, A., et al. (2009b). Candidate gene association study for noise-induced hearing Loss in two independent noise-exposed populations. *Annals of Human Genetics (2009)73,* 215-224. doi: 10.1111/j.1469-1809.2008.00499.x

Kowalski, T.J., Pawelczyk, M., Rajkowska, E., Dudarewicz, A. & Sliwinska-Kowalska, M. (2014). Genetic variants of CDH23 associated with noise-induced hearing loss. *Otology & Neurotology 35:* 358-365.

Kubisch, C., Schroeder, B. C., Friedrich, T., et al. (1999). KCNQ4, a novel potassium channel expressed in sensory outer hair cells, is mutated in dominant deafness. *Cell, 96*, 437–446.

Kundu, S., Munjal, C., Tyagi, N., Sen, U., Tyagi, A.C., and Tyagi, S.C. (2012). Folic acid improves inner ear vascularization in hyperhomocysteinemicmice. *Hear. Res. 284*,42–51. doi:10.1016/j.heares.2011.12.006

Lasisi, A. O., Fehintola, F. A., and Yusuf, O. B. (2010). Age-related hearing loss, vitamin B12, and folate in the elderly. *Otolaryngol. Head Neck Surg. 143,* 826–830. doi:10.1016/j.otohns.2010.08.031

Lim, H. H., Jenkins, O. H., Myers, M. W., et al. (1993). Detection of HSP 72 synthesis after acoustic overstimulation in rat cochlea. *Hear Res, 69,* 146–150.

Liu, Y.M., Li, X.D., Guo, X., Liu, B., Lin, A.H., & Rao, S.Q. (2010). Association between polymorphisms in SOD1 and noise-induced hearing loss in Chinese workers. *Acta Oto-Laryngologica, 2010*; 130: 477–486.

Manche, S.K., Jangala, M., Dudekula, D., Koralla, M., & Akka, J. (2018). Polymorphisms in folate metabolism genes are associated with susceptibility to presbycusis. *Life Sciences 196*(2018): 77-83. https://doi.org/10.1016/j.lfs.2018.01.015.

Martínez-Vega, R., Garrido, F., Partearroyo, T., Cediel, R., Zeisel, S.H., Martínez-Álvarez, C., et al. (2015). Folic acid deficiency induces premature hearing loss through mechanisms involving cochlear oxidative stress and impairment of homocysteine metabolism. *FASEB J. 29,* 418–432. doi:10.1096/fj.14-259283

Martínez-Vega, R., Murillo-Cuesta, S., Partearroyo, T., Varela-Moreiras, G., Varela-Nieto, I., and Pajares, M.A. (2016). Long-term dietary folate deficiency accelerates progressive

hearing loss on CBA/Ca mice. *Front. Aging Neurosci. 8:* 209. doi:10.3389/fnagi.2016.00209

Miao, L., Ji, J., Wan, L., Zhang, J., Yin, L., & Pu, Y. (2019). An overview of research trends and genetic polymorphisms for noise induced hearing loss from 2009 to 2018. *Environmental Science and Pollution Research 26*(2019): 34754-34774. https://doi.org/10.1007/s11356-019-06470-7.

Newman, D.L., Fisher, L.M., Ohmen, J., Parody, R., Fong, C.T., Frisina, S.T., Mapes, F., Eddins, D.A., Frisina, D.R., Frisina, R.D., & Friedman, R.A. (2012). GRM7 variants associated with age-related hearing loss based on auditory perception. *Hear Res. 2012, 294*(0): 125–132. doi:10.1016/j.heares.2012.08.016

Noben-Trauth, K., Zheng Q.Y., & Johnson, K.R. (2003). Association of cadherin 23 with polygenic inheritance and genetic modification of sensorineural hearing loss. *Nat Genet 35:* 21–23.

Ohlemiller, K.K., McFadden, S.L., Ding, D.L., Flood, D.G., Reaume, A.G., Hoffman, E.K., et al. Targeted deletion of the cytosolic Cu/Zn-superoxide dismutase gene (Sod1) increases susceptibility to noise-induced hearing loss. *Audiol Neurootol 1999*; 4: 237–46.

Partearroyo, T., Vallecillo, N., Pajares, M.A., Varela-Moreiras, G., & Varela-Nieto, I. (2017). Cochlear homocysteine metabolism at the crossroad of nutrition and sensorineural hearing loss. *Front. Mol. Neurosci. 10*:107. doi: 10.3389/fnmol.2017.00107.

Pawelczyk, M., Van Laer, L., Fransen, E., Rajkowska, E., Konings, A., Carlsson, P.I., Borg, E., Van Camp, G., & Sliwinska-Kowalska, M. (2009). Analysis of gene polymorphisms associated with K ion circulation in the inner ear of patients susceptible and resistant to noise-induced hearing loss. *Ann Hum Genet 73*:411–421.

Peters, L.M., Anderson, D.W., Griffith, A.J., Grundfast, K.M., San Agustin, T.B., Madeo, A.C., Friedman, T.B. and Morell, R.J. (2002) Mutation of a transcription factor, TFCP2L3,

causes progressive autosomal dominant hearing loss, DFNA28. *Hum. Mol. Genet., 11,* 2877–2885.

Samson, J., Sheeladevi, R., Ravindran, R., et al. (2007). Stress response in rat brain after different durations of noise exposure. *Neurosci Res, 57*, 143–147.

Schulze-Bahr, E., Wang, Q., Wedekind, H., et al. (1997). KCNE1 mutations cause jervell and Lange-Nielsen syndrome. *Nat Genet, 17*, 267–268.

Siemens, J., Lillo, C., Dumont, R.A., Reynolds, A., Williams, D.S., Gillespie, P.G., & Muller, U. (2004). Cadherin 23 is a component of the tip link in hair-cell stereocilia. *Nature 428:* 950– 955.

Sliwinska-Kowalska, M. & Pawelczyk, M. (2012). Contribution of genetic factors to noise-induced hearing loss: a human studies review. *Mutation Research 752*(2013): 61-65. http://dx.doi.org/10.1016/j.mrrev.2012.11.001.

Uchida, Y., Sugiura, S., Ando, F., Nakashima, T., and Shimokata, H. (2011). Hearing impairment risk and interaction of folate metabolism related gene polymorphisms in an aging study. *BMC Med. Genet. 12:* 35. doi:10.1186/14712350-12-35

Van Laer, L., Van Eyken, E., Fransen, E, Huyghe, J.R., Topsakal, V., Hendrick, J.J, Hannula, S., Maki-Torkko, E., et al. (2008). The grainyhead like 2 gene (GRHL2), alias TFCP2L3, is associated with age-related hearing impairment. *Human Molecular Genetics, 2008, 17(*2); 159–169. doi:10.1093/hmg/ddm292

Wangemann, P. (2002). K+ cycling and the endocochlear potential. *Hear Res, 165*, 1–9.

Wilanowski, T., Tuckfield, A., Cerruti, L., O'Connell, S., Saint, R., Parekh, V., Tao, J., Cunningham, J.M. and Jane, S.M. (2002) A highly conserved novel family of mammalian developmental transcription factors related to Drosophila grainyhead. *Mech. Dev., 114,* 37–50.

Yang, M., Tan, H., Yang, Q., et al. (2006). Association of hsp70 polymorphisms with risk of noise-induced hearing loss in Chinese automobile workers. *Cell Stress Chaperones, 11,* 233–239.