Contribution of genetic factors to noise-induced hearing loss

Mariola Sliwinska-Kowalska

Department of Audiology and Phoniatrics, Nofer Institute of Occupational Medicine, Lodz, Poland, marsliw@imp.lodz.pl

INTRODUCTION

It is widely accepted that noise-induced hearing loss (NIHL) is a complex disease which results from the interaction of genetic and environmental factors. Heritability might be responsible for up to 50 % of the hearing loss variability after exposure to noise.

The genetic basis of NIHL has been clearly demonstrated in animals. Mouse strains (C57BL/6J – B6) exhibiting age-related hearing loss (Ahl) were shown to be more susceptible to noise than other strains (Erway et al. 1996; Davis et al. 2001). Also, several knockout mice including SOD1-/- (Ohlemiller et al. 1999); GPX1-/- (Ohlemiller et al. 2000); PMCA2 -/- (Kozel et al. 2002) and CDH23+/- (Holme & Steel 2004) were shown to be more sensitive to noise than the wild-type littermates.

Over the last 10 years a great increase in association studies that were trying to identify the susceptibility genes for NIHL in humans was also observed. Tens and hundreds of Single Nucleotide Polymorphisms (SNPs) of different genes that are known to play a functional and morphological role in the inner ear were screened. SNPs are common point mutations in the genome (occurring every 100 – 300bp), and their genotyping is believed to be a successful tool in analysis of the genetic background of complex diseases, like NIHL. In such studies, disease susceptibility allele is expected to occur more often in susceptible group than in a resistant one.

METHODS

The aim of this paper was to overview human association study results on gene polymorphisms in human populations exposed to noise and indicate the first potential susceptibility genes for NIHL. The review includes papers published over the last 10 years in English. Eleven most crucial human papers were identified by literature search of accessible medical and other databases (PubMed, Embase, Scopus, Bio-Med Central, Web of Science).

RESULTS

So far, the most promising results were obtained for genes involved in the inner ear potassium ion recycling (van Laer et al. 2006; Pawelczyk et al. 2009) and heat shock protein genes (HSP70) (Yang et al. 2006; Konings et al. 2009a), because they were replicated in the independent populations, and were sufficient in size to yield high power for the detection of a causative allele. The other genes of interest are oxidative stress genes (Rabinowitz et al. 2002; Fortunato et al. 2004, Carlsson et al. 2005; Konings et al. 2007). Lately, the significance of genetic variation in NIHL development has been also shown for otocadherin 15 and myosin 14 genes (Konings et al. 2009b).

Potassium-recycling genes

K^+ recycling is of great importance for the process of hearing. The ions are secreted into the endolymph by the stria vascularis, enter the hair cells through apical mechanosensitive K^+ channels and leave these cells via their basolateral membrane, then migrate through supporting cells and fibrocytes toward the stria vascularis using a network of gap junctions. K^+ recycling genes seem to be very good candidate genes for susceptibility to NIHL, what is supported by the fact that mutations in K^+ channel genes in the inner ear often lead to hearing loss. Mice deficient for *KCNE1*, *KCNQ1* or *SLC12A2* have collapsed endolymphatic spaces, while in humans several mutations in *KCNQ1* or *KCNE1* potassium channel subunits lead to pathological cardiac and auditory phenotypes.

A total of 10 genes putatively involved in potassium ions recycling in the inner ear were examined, namely five connexin genes: Cx26 (*GJB2*), Cx30 (*GJB6*), Cx30.3 (*GJB4*), Cx31 (*GJB3*) Cx32 (*GJB1*), four potassium channels or subunits (*KCNJ10*, *KCNQ4*, *KCNE1*, *KCNQ1*) and one Na+/2Cl-/K+ co-transporter in large populations of Swedish and Polish workers. Allele, genotype and haplotype frequencies were compared between noise-susceptible and resistant groups (103 susceptible and 114 noise-resistant workers selected from over 1200 subject database in Sweden, and 119 susceptible and 119 resistant workers selected from the subpopulation of over 3,000 subject database in Poland) (Carlsson et al. 2005; van Laer et al. 2006; Pawelczyk et al. 2009).

In the Swedish sample, significant differences were observed for 3 SNPs of *KCNE1*, one SNP for *KCNQ1*, and one SNP of *KCNQ4*, suggesting that these are first defined susceptibility genes for NIHL (Van Lear et al. 2006). In the Polish sample, which comprised substantially more SNPs (99 vs. 35 in the Swedish sample), a significant associations were found in 7 out of 10 genes (*KCNE1*, *KCNQ4*, *GJB1*, *GJB2*, *GJB4*, *KBNJ10*, *KCNQ1*) (Pawelczyk et al. 2009).

The most interesting results were obtained for *KCNE1* and *KCNQ4*, as the authors replicated associations for the same SNPs that were previously reported in a Swedish sample set (rs2070358 and Q455H, respectively). The direction of genetic trends for *KCNE1* was the same in both populations, but opposite for *KCNQ4*, questioning the replication for the latter gene. Since the analysis of the linkage disequilibrium (LD) pattern within the region of *KCNE1* demonstrated that rs2070358 and D85N were not in LD with each other, certainly *KCNE1* can be considered as NIHL susceptibility gene. In Polish sample set a significant association was also found for KCNQ1; however, in different SNPs than in the Swedish population. However, taking into account that different SNPs association in different populations but within the same gene, may be regarded as a replication, it seems likely that, in addition to *KCNE1*, also *KCNQ1* is truly a susceptibility gene for NIHL.

KCNE1 and *KCNQ1* are functionally linked. *KCNE1* encodes a K^+-channel beta subunit. It requires coexpression of an alfa-subunit, usually *KCNQ1*, to generate a functional K^+ channel. KCNQ1/KCNBE1 channels are present at the marginal cell membrane of stria vascularis and play a major role in cardiac as well as inner ear function. *KCNE1* mutations cause long QT syndromes (the autosomal recessive Jervell and Lange-Nielsen syndrome or the autosomal dominant Romano Ward syndrome). It has been shown that in the inner ear, the presence of *KCNE1*-p.85N variant might

lead to slightly higher K^+ concentrations, what would render the organ of Corti more sensitive to noise damage (van Laer et al. 2006).

Hsp70 genes

Heat-shock proteins (HSPs) form a group of conserved proteins assisting in synthesis, folding, assembly and intracellular transport of many other proteins. HSPs are ubiquitously expressed in the body cells under physiological and pathological conditions. Their expression increases under stressful condition, including noise exposure. When first induced by exposure to moderate sound levels, they can protect the ear from excessive noise exposure. Three genes are responsible for HSPs synthesis, *HSP70-1*, *HSP70-2* and *HSP70-hom*. The genes are heat inducible, except the last one.

Variations in HSP70 genes were shown to be associated with susceptibility to NIHL and these results were replicated in three independent populations, Chinese, Swedish and Polish (Yang et al. 2006; Konings et al. 2009a). In Chinese population of 194 autoworkers, no statistically significant difference was shown in the genotype and allele distribution among 93 subjects who developed hearing loss comparing with 101 subjects without hearing deficit. However, assuming that SNP may not be sufficiently informative in complex disease, haplotype analysis was preformed. It showed that two haplotypes among six were significantly more frequent in the NIHL group vs. control. Using similar methodology and data analysis for the same gene polymorphisms, these findings were confirmed in the groups of 206 Swedish and 238 Polish workers (group selection has been described above). One SNP, rs2227956 in *HSP70-hom*, resulted in a significant association with NIHL in both European sample sets. Moreover, one haplotype (GAC) was also associated with NIHL in both these sample sets, and one other 9 (CGT) in Swedish population. Haplotype GAC showed protective effect in both samples, with twofold decreased odds of developing NIHL.

The comparison of putative haplotypes among Asian and European populations revealed that haplotypes significantly associated with NIHL in Chinese people (GGC and GGT) were infrequent or absent in the Swedish and Polish population. However, it does not exclude a true association of this gene polymorphisms with susceptibility to noise, and may be explained by the ethnic difference between the sample sets.

Several other studies suggested that *HSP70* polymorphisms can be associated with many other diseases, including Parkinson's disease, Crohn disease or ischemic stroke, among others. HSP70 protein may also play a role in autoimmune inner ear disease.

Oxidative stress genes

Oxidative stress plays a major role in the pathomechanisms of NIHL. A local prolonged release of free radicals (reactive oxygen and nitrogen species) after noise overexposure may result in cochlear epithelium damage, particularly if the antioxidant defense system is not efficient enough to neutralize them.

There are two groups of antioxidant enzymes that are active in the cochlea. The first group comprises enzymes involved in glutathione metabolism, including glutathione S-transferase (GST), glutathione peroxidase (GPX1), and glutathione reductase (GSR). GST classes comprise *GSTM1* and *GSTT1* genes which show great genetic variability in humans. Up to 50 % of the Caucasian population are null genotypes for

GSTM1 gene, and 25-40 % of the Caucasian population are null genotypes for the GSMT1 gene. The second class of antioxidant enzymes includes the enzymes involved in the breakdown of superoxide anions and hydrogen peroxide (catalase – *CAT*, superoxide dismutase 1, Cu/Zn – *SOD1*, superoxide dismutase 2, mitochondrial – *SOD2*, serum paraoxonase/arylesterase 2 – *PON2*).

The results of the study on association between variations in oxidative stress genes snf susceptibility to NIHL are equivocal. First it was shown that *GSTM1* null individuals exposed to noise had lower amplitudes of high frequency otoacoustic emission comparing to individuals possessing the gene (Rabinowitz et al. 2002). Another study of a limited sample set suggested that *SOD2* and *PON2* gene polymorphisms may be associated with NIHL (Fortunato et al. 2004). However, all these associations might be incidental, due to insufficient power.

In much more comprehensive study performed in Swedish workers (103 susceptible to noise and 114 resistant to noise workers selected from over 1,200 subject database), none of seven oxidative stress genes, namely *GSTM1*, *GSTT1*, *CAT*, *SOD*, *GPX*, *GSR* and *GSTP1*, was shown to be a susceptibility gene for NIHL (Carlsson et al. 2005). However, the same authors have shown that the effect of smoking on susceptibility to NIHL is dependent on the presence of the *GSTM1* deletion, suggesting a substantial interaction of genes and environmental factors in NIHL development (Carlsson et al. 2005).

Similarly, association study in two large independent populations (Swedish and Polish, described above) indicates that the effect of Catalase (*CAT*) gene polymorphism on susceptibility to NIHL may only be detected when noise exposure level is taken into account (Konings et al. 2007). Moreover, the same genotype can have a differential effect on the susceptibility to NIHL, depending on the noise exposure level.

More recent studies support the role of oxidative stress gene polymorphisms in the development of NIHL. *SOD2* SNP in the mitochondrial targeting sequence was shown to be associated with noise-induced hearing loss in Chinese workers, and again this effect was enhanced by higher levels of noise exposure (Liu et al. 2010).

Also, double blind, crossover study in 53 male workers treated with N-Acetyl-cysteine support the hypothesis that individuals carrying all genotypes with *GSTT1* null, *GSTM1* null, and *GSTP1* Ile(105)/Ile(105) are more susceptible to NIHL (Lin et al. 2009). On the other hand, the ototoxicity of aminoglycosides, which seem to involve similar oxidative stress mechanisms, was shown to be independent of *GSTM1* and *GSTT1* gene polymorphisms (Palodetto et al. 2010).

Other genes

Lately, an extended analysis of 644 SNPs in 53 candidate genes was performed in two independent (Swedish and Polish) populations. The positive associations were shown for two genes, one encoding protocadherin 15 (*PCDH15*), and the other encoding myosin 14 (*MYH14*) (Konings et al. 2009b). One SNP in *PCDH15* resulted in significant associations in both populations, and two SNPs in *MYH14* resulted in positive association in the Polish sample set and significant interaction with noise exposure level in the Swedish sample set.

Cadherins, namely cadherin 23 and protocadherin 15 are the molecules that form tip links between sensory hair cells of the cochlea, and are essential for the mechanoelectrical transduction (Sakaguchi et al. 2009). It was shown that mutation in *Cdh 23* disrupted stereocilia organization on hair cells leading to deafness and vestibular dysfunction in waltzer mice; the 753A variant of his gene was correlated with susceptibility to noise-induced hearing loss (Noben-Trauth et al. 2003). In humans, *PCDH15* and *CDH23* gene mutations are associated with both syndromic and non-syndromic hearing loss (DFNB23, Usher syndrome type 1F, and Usher syndrome type 1D, respectively).

MYH14 encodes one of the proteins of the myosin superfamily. They are actin-dependent motor proteins regulating cochlear hair cells motility and polarity. Mutation in *MYH14* results in autosomal dominant hearing impairment in humans (DFNA4).

CONCLUSIONS

Up to now, association studies on susceptibility genes for NIHL were performed based on candidate gene approach. It was shown that several gene polymorphisms are probably involved in determining susceptibility to NIHL. In establishing the role of some of them, searching for the interaction between gene variations and environmental factors is necessary. It mainly regards noise exposure, since the mechanisms of cochlear damage is straightly related to its level and time of exposure.

To confirm the role of the gene in the development of NIHL, the replication of the results in independent population sample sets is mandatory. But, taking into account the ethnic differences the replication at the level of gene, and not necessarily the polymorphism or haplotype, is satisfactory. Due to difficulties in replicating the results on one hand, and the development of high-throughput genotyping methods along with the growing databases of SNPs on the other one, a logical next step for research on genetics of NIHL is Whole Genome Association Studies. Genomewide Single-Nucletide-Polymorphism association studies are optimal approaches for determining whether major genetic association exist in diseases with high heritability like NIHL. The development of the methods allowing to genotype hundreds and thousands of SNPs in a single array will undoubtedly lead toward identification of new NIHL susceptibility genes.

Detection of genetic factors contributing to the development of noise-induced hearing loss will allow for a better understanding of NIHL pathophysiology and it will indicative direction for further analysis of this condition. Identification of susceptibility genes may lead to the development of genetic tests which would allow to personalize treatment – gene therapy is a possible approach, but also applying specific medications might be advisable. It can also be helpful in identifying the population at high risk along with allowing for better hearing protection in predisposed individuals.

REFERENCES

Carlsson PI, van Laer L, Borg E et al. (2005). The influence of genetic variation in oxidative stress genes on human noise susceptibility. Hear Res 202: 87-96.

Davis RR, Newlander JK, Ling X et al. (2001). Genetic basis for susceptibility to noise-induced hearing loss in mice. Hear Res 155: 82-90.

Erway LC, Shiau YW, Davis RR et al. (1996). Genetics of age-related hearing loss in mice. III. Susceptibility of inbred and F1 hybrid strains to noise-induced hearing loss. Hear Res 93: 181-187.

Fortunato G, Marciano E, Zarrilli F (2004). Paraoxonase and superoxide dismutase gene polymorphisms and noise-induced hearing loss. Clin Chem 50: 2012-2018.

Holme RH, Steel KP (2004). Progressive hearing loss and increased susceptibility to noise-induced hearing loss in mice carrying a Cdh23 but not a Myo7a mutation. J Assoc Res Otolaryngol 5: 66-79.

Konings A, van Laer L, Pawelczyk M et al. (2007). Association between variations in CAT and noise-induced hearing loss in two independent noise-exposed populations. Hum Mol Genet 16: 1872-1883.

Konings A, van Laer L, Michel S et al. (2009a). Variations in HSP70 genes associated with noise-induced hearing loss in two independent populations. Eur J Hum Genet 17: 329-335.

Konings A, van Laer L, Wiktorek-Smagur A et al. (2009b). Candidate gene association study for noise-induced hearing loss in two independent noise-exposed populations. Ann Hum Genet 73: 215-224.

Kozel PJ, Davis RR, Krieg EF et al. (2002). Deficiency in plasma membrane calcium ATPase isoform 2 increases susceptibility to noise-induced hearing loss in mice. Hear Res 164: 231-239.

Lin CY, Wu JL, Shih TS et al. (2009). Glutathione S-transferase M1, T1, and P1 polymorphisms as susceptibility factors for noise-induced temporary threshold shift. Hear Res 257: 8-15.

Liu YM, Li XD, Guo X et al. (2010). SOD2 V16A SNP in the mitochondrial targeting sequence is associated with noise induced hearing loss in Chinese workers. Dis Markers 28: 137-147.

Noben-Trauth K, Zheng QY, Johnson KR (2003). Association of cadherin 23 with polygenic inheritance and genetic modification of sensorineural hearing loss. Nat Genet 35: 21-23.

Ohlemiller KK, McFadden SL, Ding DL et al. (1999). Targeted deletion of the cytosolic Cu/Zn-superoxide dismutase gene (Sod1) increases susceptibility to noise-induced hearing loss. Audiol Neurootol 4: 237-246.

Ohlemiller KK, McFadden SL, Ding DL et al. (2000). Targeted mutation of the gene for cellular glutathione peroxidase (Gpx1) increases noise-induced hearing loss in mice. J Assoc Res Otolaryngol 1: 243-254.

Palodetto B, Postal M, Grignoli CR et al. (2010). Influence of glutathione s-transferase on the ototoxicity caused by aminoglycosides. Hear Res 269: 42-47.

Pawelczyk M, van Laer L, Fransen E et al. (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.

Rabinowitz PM, Pierce Wise J, Hur Mobo B et al. (2002). Antioxidant status and hearing function in noise-exposed workers. Hear Res 173: 164-171.

Sakaguchi H, Tokita J, Müller U et al. (2009). Tip links in hair cells: molecular composition and role in hearing loss. Curr Opin Otolaryngol Head Neck Surg 17: 388-393.

van Laer L, Carlsson PI, Ottschytsch N et al. (2006). The contribution of genes involved in potassium recycling in the inner ear to noise-induced hearing loss. Hum Mutat 27: 786-795.

Yang M, Tan H, Zheng JR et al. [Association of cadherin CDH23 gene polymorphisms with noise induced hearing loss in Chinese workers]. Wei Sheng Yan Jiu 2006; 35(1): 19-22 [article in Chinese].