

# Noise stresses the junctions to deaf

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**Sitting in the rainforest in Costa Rica, you can hear rain, rushing water, howling monkeys, birds and crickets. All are in abundance and although they are tantalizing to all your senses, your sense of hearing picks up what you cannot see with your eyes or feel with your hands.**

But harming your sense of hearing are other frequent sounds: airplanes, trucks and unusually loud music from the club in your hotel. Accumulating over time, particularly if your genome contains a genetic mutation in the vezatin gene, these harmful sounds may lead to noise-induced hearing loss (NIHL). Research led by Christine Petit and published in this issue of EMBO Mol Med, demonstrates that vezatin, localized to the junctions between cells of the cochlea, is required to protect hair cells against the damages of NIHL due to mechanical stress. Even without exposure to excessive noise, a mutation in vezatin will lead to hearing loss, though the nature of this loss is progressive and takes more time. Therefore, vezatin is still essential for maintaining the integrity of the junctions due to long-term mechanical stress imposed by sound over time. Vezatin is an adherens junction protein, an essential component of many cells, including those of the inner ear. All of the above and more have been elegantly described by Bahloul et al through the use of a

conditional mouse mutant lacking vezatin, specifically in the inner ear.

The inner ear contains two sensory organs: the auditory organ known as the cochlea and the balance organ, the vestibule (reviewed in Martin, 2003). Hair cells within the organ of Corti of the spiral shaped cochlea and within the saccule, utricle and cristae ampullaris of the three semicircular canals of the vestibule provide us with the ability to hear and balance ourselves. In order for mechanical stimulation to be transformed into an electrical signal, sound waves travel through the auditory canal and vibrate the tympanic membrane. This causes the movement of the three ossicles of the middle ear, with the stapes inducing vibration of the endolymph in the inner ear. This in turn leads to deflection of the stereocilia that elicits the opening of mechanotransduction channels and thus influxes of  $K^+$  and depolarization of the hair cells. This cascade, initiated by sound, is associated with mechanical stress in some of the components of the organ of Corti. As in other tissues, it is the adherens junction (AJ) that is responsible for maintaining tissue integrity and protection against mechanical stress. Vezatin is one of the proteins which along with claudins, ZO1 and  $\alpha$ - and  $\beta$ -catenin, forms these junctions between hair cells and supporting cells (Nunes et al, 2006). Based on the Bahloul et al study, vezatin appears to be an essential component in this protective mechanism.

Bahloul et al took advantage of the relative ease of eradicating the expression of a gene in a particular tissue using the Cre-loxP recombination system (Kilby et al, 1993; Yu & Zuo, 2009). Vezatin 'floxed' mice (*Vezt<sup>fl/fl</sup>*) were crossed with trans-

genic mice expressing the Cre recombinase driven by the *Prestin* promoter (*Vezt<sup>fl/fl</sup>;Prestin<sup>Cre</sup>*). Both vezatin-floxed mice with normal expression of vezatin and those with a specific deletion of vezatin in the inner ears suffered an increase in hearing thresholds and damage to the hair cells after 1 minute exposure to a broadband continuous noise (flat spectrum in the 2–50 kHz range) at an intensity of 105 dB SPL. This is equivalent to the sound of a tractor held at the base of your ear. This conclusion was reached by examining the hearing levels and organ of Corti morphology 30 minutes after exposure to noise. However, 8 days later, the hearing of vezatin-floxed mice resumed to normal levels and morphology of hair cells, whereas the mutant mice still had impaired hearing and morphology (Fig 1). Mutant mice that had not been exposed to excessive noise were also examined at this same age and were found to have normal hearing and inner ear morphology. Therefore, the deficit due to exposure to noise was sustainable in mutant mice only.

» **Vezatin protection of tight junctions against mechanical stress.** «

Over the years, not only have primary mutations been discovered in genes, leading to deafness, but the interaction between the proteins they encode have been found to form networks. The most well-characterized network is that of the Usher interactome, where as many as eight proteins, all leading to the combined deafness–blindness Usher syn-

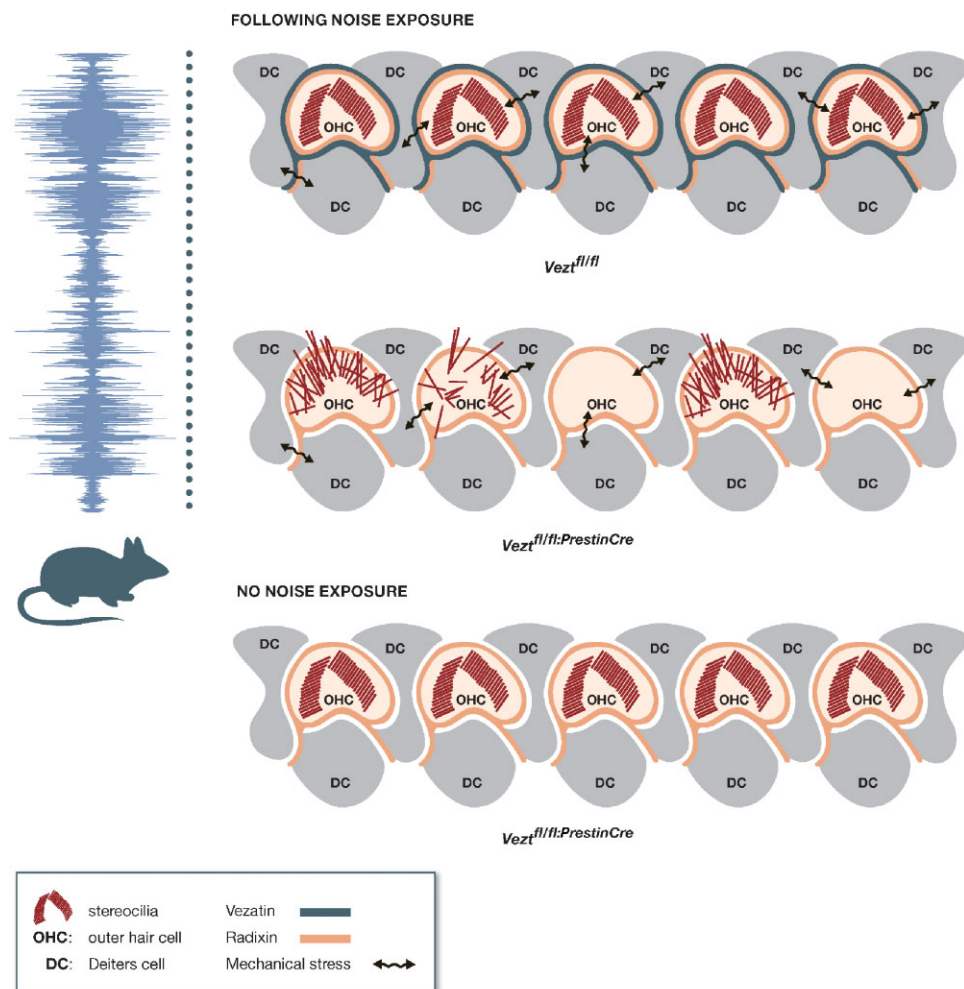
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**Figure 1.** Mice were exposed to excessive noise, which subjected the hair cells and supporting cells of the inner ear to mechanical stress affecting the tight adherens junctions (TAJs). This noise was equivalent to the sound of a tractor held close to the ear. The cells of mice with normal levels of vezatin ( $Vezt^{fl/fl}$ ) survived this exposure well after 8 days. Mice lacking vezatin ( $Vezt^{fl/fl};PrestinCre$ ), however, lost a portion of their hair cells and those remaining had abnormal stereocilia, the actin-rich projections on the apical surface of hair cells. Although mice without vezatin eventually lost their hearing due to age-related hearing loss (ARHL), their hair cells were intact at 7 weeks when not exposed to noise. Radixin was found to interact with vezatin in the TAJs and may facilitate the joining of vezatin and actin at these junctions, providing the mechanism for protecting and strengthening the cell junctions. Figure designed and produced by Amiel Dror, Tel Aviv University.

drome, interact with one another (reviewed in Kremer et al, 2006; Saihan et al, 2009). The complexity of interactions is remarkable, although one mutation in just one of these genes is enough to destroy the cascade of events leading to healthy cochleas and retinas. Here too, the authors have discovered the beginning of a network of interactions. Previously, vezatin was shown to interact with myosin VIIa (Kussel-Andermann et al, 2000). Now, experiments reveal that

vezatin interacts with Radixin, another actin-binding protein of the ERM family and associated with the DFNB24 form of human hearing loss (Khan et al, 2007). It is this association that may provide a clue to the mechanism of vezatin protection of tight junctions against mechanical stress: through its binding to actin, vezatin strengthens these junctions. Without vezatin, the junctions are weaker and more susceptible to damage by noise and/or ageing.

## » Genetic characterization of NIHL is in its infancy. «

What is the broader implication for this work? The molecular genetic characterization of NIHL is in its infancy. The link between vezatin, mechanical stress on the inner ear tight junctions, and NIHL is a compelling one and paves the way for more research in this area. Not only is the list of genes implicated in this complex

form of hearing loss short, but the molecular basis of NIHL is largely unknown. Traditionally, this has been studied in animal models, but over the last few years, several studies have been performed using audiometric data from populations of noise-exposed workers, in conjunction with genotyping of single nucleotide polymorphisms (SNPs). There seems to be no association between the most prevalent connexin 26 (*GJB2*) mutation, 35delG and NIHL, at least in a study performed in a population of workers exposed to noise (Van Eyken et al, 2007). Oxidative stress may be a complicating factor in NIHL because an association was found for a few genes that protect against oxidative stress (reviewed in Konings et al, 2009). One of the studies found an association with SNPs in genes involved in potassium-recycling; electrophysiological recordings in transfected cell cultures suggested abnormalities in *KCNQ1/KCNE1*-mutated channels relative to those of wild-type channels (Van Laer et al, 2006). Although far from being an exhaustive list, the work done so far is promising but more studies are needed to replicate these results. Now the research described with vezatin is a major contributor to creating a genuine link between the molecular basis of NIHL and the underlying mechanism.

Are there common links between NIHL and age-related hearing loss (ARHL), the most prevalent form of hearing impairment? The work by Bahloul et al. suggests that there are common mechanisms leading to both. Indeed,

aging *Vezt<sup>fl/fl;PrestinCre</sup>* mice eventually suffered from hearing loss, with no early exposure to excessive noise. ARHL is a major economic and social burden on society, while NIHL is a close second. In both cases, both genetic and environmental factors contribute. As our ageing population grows and constant exposure to deleterious noise (how loud do your kids play their MP3?) increases, we are threatened with a vastly expanding population that cannot function optimally through their sense of hearing.

Finally, where is all this work leading to? There is great promise and hope that determining the underlying mechanisms behind complex forms of hearing loss will lead to therapeutic options, including gene- and stem cell-based therapies (Martinez-Monedero et al, 2007; Raphael et al, 2007). Finding ways to lower 'stress' in the junctions of our inner ears will certainly help towards reducing deafness.

The author declares that she has no conflict of interest.

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