**Data input form: MouseAGE Database**

*Author*: Silvia Murillo-Cuesta, Isabel Varela-Nieto

*Institution*: Centro de Investigación Biomédica en Red (CIBER); Agencia Estatal Consejo Superior de Investigaciones Científicas (CSIC)

*Country*: Spain

*Date submitted*: **April 16th 2018**

*Preferred acknowledgement*:

*Summarise supporting data attached*: Auditory phenotype of C57BL/6J mouse strain, a standard animal model to study age-related and noise-induced hearing loss

**Ageing / disease:** Age-related hearing loss or presbycusis. Noise-induced hearing loss.

**Model:** Inbred mouse strain C57BL/6J, which has the *Cdh23$^{ahl}$* allele (Johnson et al, 2000).

**Clinically relevant? (Yes or No):**Yes

**Explanation of why clinically relevant or not:** *Cdh23* encodes a cell-cell adhesion glycoprotein expressed in the sensory epithelium of the inner ear and is required for stereocilium bundle formation and function. The *Cdh23$^{ahl}$* allele is defined by the presence of an A rather than a G at the last position of the 7th coding exon (Noben-Trauth et al., 2003). This allele causes an increased frequency of in-frame exon skipping and is common to at least 10 inbred strains with increased susceptibility to ARHL (Johnson et al, 2000). Mutant alleles of the human orthologue CDH23 cause recessive deafness (DFNB12) and Usher's syndrome (USH1D).

**Characteristics, timing of appearance of phenotype and phenotypic tests used (attach supporting data when unpublished if available):**

C57BL/6J strain begins to display elevated hearing thresholds (measured with Auditory Brainstem Response test) at 3-6 months of age, starting in the high frequencies, then the low, before leading to a profound hearing loss across all frequencies. Curiously, they do not show a decline in endocochlear potential (Ohlemiller et al 2006).

Hearing loss is associated with a progressive loss of both inner and outer hair cells in an age-dependent manner, from the base to the apex of the cochlear spiral. Outer hair cell loss is observed at the base of the cochlea by 3 months of age. Between 3 and 7 months of age, loss of inner hair cells becomes evident at the basal turn. By 7 months of age, loss of spiral ganglion cells is also evident and is consistent with a neuronal degeneration secondary to inner hair cell loss (Hequembourg et al, 2001). Additionally, age-related changes are also seen in the spiral ligament and stria vascularis consisting of fibrocyte loss and thinning, respectively (Ohlemiller, 2006).

C57BL/6J mice are also especially vulnerable to noise and a model to study noise-induced hearing loss (Ohlemiller et al., 2000 and 2002; Ohlemiller and Gagnon, 2007; Sanz et al., 2015).

C57BL/6J mice have also been employed to investigate the role of melanin pigment in the stria vascularis, as a protective factor against ARHL, comparing them with the co-isogenic substrain C57BL/6J-Tyrc-2J, which does not produce any strial melanin due to a naturally occurring mutation in the tyrosinase locus. Assessment of these mice showed identical rates of hearing loss and sensory cell loss. However, aged (>2 years) C57BL/6J-Tyrc-2J mice have reduced endocochlear potentials

compared to the pigmented C57BL/6J mice, and this correlates with a significantly reduced strial thickness in the albino mice due to a net loss of marginal cells (Ohlemiller et al., 2009). The authors propose that absence of melanin promote age-related strial marginal cell loss through a reduced ability to scavenge reactive oxygen species (ROS).

**If applicable, Intervention(s) performed (type, dose, route of administration, frequency):**

Nutritional approach: long-term ω3 supplementation partially prevents progression of hearing loss in C57BL/6J mice. This effect is mainly exerted through maintenance of IGF-1 signaling and the balance between proinflammatory and anti-inflammatory cytokines. Additionally, the increased Hcy elimination through transsulfuration observed 10 month-old C57 mice cochleae is compensated by the ω3 diet through increased BHMT remethylation to preserve the flux through the methionine cycle and the protein homocysteinylation levels within normality. However, age-related activation of the p38 stress pathway is not prevented, and hence, changes in protein expression leading to hearing loss are still observed (Martínez-Vega et al., 2015).

Genetic approach: CRISPR/Cas9-mediated HDR has been successfully utilised to efficiently correct the *Cdh23*$^{ahl}$ allele in C57BL/6NTac mice, and rescue the associated auditory phenotype (Mianné et al., 2016).

Pharmacologic approach: Several antioxidant and anti-inflammatory treatments has been evaluated in C57BL/6 mice exposed to noise, including resveratrol (Xiong et al., 2017), dexamethasone (Han et al., 2015)or TGFb1 inhibitors (Murillo-Cuesta et a., 2015).

**Phenotype post-treatment (include null or negative results and attach supporting data separately):**

Nutritional approach (Martínez-Vega et al., 2015): Ten month-old C57BL/6J mice feeded with w3 supplemented-diet showed significant decreases in the ABR thresholds at several frequencies as compared to the standard diet group. Precisely, ABR thresholds in response to 4-, 8- and 40-kHz frequencies declined 27 dB SPL (P<.001), 16 dB SPL (P=.02) and 17 dB SPL (P=.027), respectively. Age-related changes in the ABR waves include a decrease in their amplitudes together with an increase on their latencies.

Genetic approach (Mianné et al., 2016): By 24 and 36 weeks of age the *Cdh23*$^{ahl/ahl}$ mice have significantly elevated hearing thresholds (>60 and >80 dB SPL, respectively) at the highest frequency tested (32 kHz), whereas the *Cdh23*$^{ahl/753A>G}$ mice have hearing thresholds within the normal range (15–35 dB SPL) at all frequencies tested (8, 16 and 32 kHz).By 36 weeks of age the *Cdh23*$^{ahl/ahl}$ mice show loss of OHC stereocilia bundles in the base, whereas the *Cdh23*$^{ahl/753A>G}$ mice do not show loss of IHC or OHC bundles, in any region of the cochlea.

Pharmacologic approach: Antioxidant and anti-inflammatory treatments reduce threshold shifts after noise insult in C57BL/6J mice.

**References (list below):**

*Han MA, Back SA, Kim HL, Park SY, Yeo SW, Park SN. Therapeutic Effect of Dexamethasone for Noise-induced Hearing Loss: Systemic Versus IntratympanicInjection in Mice. Otol Neurotol. 2015 Jun;36(5):755-62.*

*Hequembourg S, Liberman MC: Spiral ligament pathology: a major aspect of age-related cochlear degeneration in C57BL/6 mice. J Assoc Res Otolaryngol 2001;2:118-129.*

*Johnson KR, Zheng QY, Erway LC: A major gene affecting age-related hearing loss is common to at least ten inbred strains of mice. Genomics 2000;70:171-180.*

*Martínez-Vega R, Partearroyo T, Vallecillo N, Varela-Moreiras G, Pajares MA, Varela-Nieto I. Long-term omega-3 fatty acid supplementation prevents expression changes in cochlear homocysteine metabolism and ameliorates progressive hearing loss in C57BL/6J mice. J Nutr Biochem. 2015 Dec;26(12):1424-33.*

*Mianné J, Chessum L, Kumar S, et al. Correction of the auditory phenotype in C57BL/6N mice via CRISPR/Cas9-mediated homology directed repair. Genome Medicine. 2016;8:16. doi:10.1186/s13073-016-0273-4.*

*Murillo-Cuesta S, Rodríguez-de la Rosa L, Contreras J, Celaya AM, Camarero G, Rivera T, Varela-Nieto I. Transforming growth factor ß1 inhibition protects from noise-induced hearing loss. Front Aging Neurosci. 2015 Mar 20;7:32.*

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

*Ohlemiller KK, Gagnon PM. Genetic dependence of cochlear cells and structures injured by noise. Hear Res. 2007 Feb;224(1-2):34-50.*

*Ohlemiller KK, Rice ME, Lett JM, Gagnon PM: Absence of strial melanin coincides with age-associated marginal cell loss and endocochlear potential decline. Hear Res 2009;249:1-14.*

*Ohlemiller KK, Wright JS, Heidbreder AF. Vulnerability to noise-induced hearing loss in 'middle-aged' and young adult mice: a dose-response approach in CBA, C57BL, and BALB inbred strains. Hear Res. 2000 Nov;149(1-2):239-47.*

*Ohlemiller KK: Contributions of mouse models to understanding of age- and noise-related hearing loss. Brain Res 2006;1091:89-102.*

*Sanz L, Murillo-Cuesta S, Cobo P, Cediel-Algovia R, Contreras J, Rivera T, Varela-Nieto I, Avendaño C. Swept-sine noise-induced damage as a hearing loss model for preclinical assays. Front Aging Neurosci. 2015 Feb 16;7:7.*

*Xiong H, Ou Y, Xu Y, Huang Q, Pang J, Lai L, Zheng Y. Resveratrol Promotes Recovery of Hearing following Intense Noise Exposure by Enhancing Cochlear SIRT1 Activity. Audiol Neurootol. 2017;22(4-5):303-310.*