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Differential effects of *Cdh23*^{753A} on auditory and vestibular functional aging in C57BL/6J mice

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ABSTRACT

The C57BL/6J (B6) mouse strain carries a cadherin 23 mutation (*Cdh23*^{753A}, also known as *Ahl*), which affects inner ear structures and results in age-related hearing loss. The B6.CAST strain harbors the wild type *Cdh23* gene, and hence, the influence of *Ahl* is absent. The purpose of the present study was to characterize the effect of age and gender on gravity receptor function in B6 and B6.CAST strains and to compare functional aging between auditory and vestibular modalities. Auditory sensitivity declined at significantly faster rates than gravity receptor sensitivity for both strains. Indeed, vestibular functional aging was minimal for both strains. The comparatively smaller loss of macular versus cochlear sensitivity in both the B6 and B6.CAST strains suggests that the contribution of *Ahl* to the aging of the vestibular system is minimal, and thus very different than its influence on aging of the auditory system. Alternatively, there exist unidentified genes or gene modifiers that serve to slow the degeneration of gravity receptor structures and maintain gravity receptor sensitivity into advanced age.

1. Introduction

The inner ear houses the sensory organs for hearing and balance. Dysfunction in any of the inner ear end organs or their central pathways can cause hearing and/or vestibular impairment. Age-related hearing loss (ARHL) is the most common type of hearing impairment in humans, affecting 50% of the population by age 80 (Gorlin et al., 1995; Morton, 1991). The anatomical and physiological mechanisms of auditory aging have been extensively studied in both humans and animal models. However, relatively little has been done to investigate the role of age and predisposing factors (such as gender and genetic background) on vestibular function in spite of evidence that 3.4% of the US adult population (6.2 million people) suffers from chronic dizziness and/or imbalance (Hoffman and Sklare, 2003), 24% of people older than 72 years in the United States have suffered an episode of dizziness lasting for at least a month (Tinetti et al., 2000), 30% of people older than 65 years in the UK have dizziness (Colledge et al., 1994) and as many as 7 million people per year seek care for disequilibrium and/or vertigo.

Studies of the effect of age on vestibular function have typically used indirect measures such as the vestibulo-ocular reflex (VOR), optokinetic response, the otolith-ocular reflex, visual-vestibular responses, and tests of posture to infer peripheral vestibular status (Baloh et al., 1993; Enrietto et al., 1999; Furman and Redfern, 2001; Goebel, 2001; Paige, 1992, 1994; Shiga et al., 2005). Reported findings include decreased VOR gain, increased phase lead, decreased ability to suppress VOR with vision, less shortening of the VOR time constant by postrotary head tilt, and lower optokinetic response slow-phase velocity saturation.

Studies using a direct measure of peripheral vestibular function (vestibular sensory evoked potentials [VsEPs]), in a variety of inbred mouse strains, highlight the importance of genetic background for gravity receptor function (e.g., Jones et al., 2005, 2006). Furthermore, these studies suggest that a genetic predisposition for a functional deficit in one inner ear sensory modality does not obligate functional loss in the other modality (e.g., Jones et al., 2005, 2006; Lee et al., 2013; Zhao et al., 2008).

The C57BL/6J (B6) mouse has been extensively studied and is a well-established model for ARHL (Bartolome et al., 2002; Henry, 2002; Hequembourg and Liberman, 2001; Johnson et al., 1997;

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McFadden et al., 2001; Spongr et al., 1997). To date, at least 10 quantitative trait loci that contribute to ARHL have been identified in mice (Latoche et al., 2011; Noben-Trauth and Johnson, 2009). The *Ahl* mutation (also known as *Cdh23*^{753A}) was the first mutation discovered in association with ARHL and appears to be the most common ARHL mutation (Johnson et al., 1997; Noben-Trauth et al., 2003). *Ahl* is a recessive, single nucleotide mutation at 753 (G≥A) on the *Cdh23* gene on mouse chromosome 10 (Noben-Trauth et al., 2003). *Cdh23* encodes cadherin 23, a protein necessary for inner ear development and maintenance of sensory cell structures such as stereociliary tip links (Kazmierczak et al., 2007; Siemens et al., 2004; Söllner et al., 2004) kinocilial and transient lateral links (Lagziel et al., 2005; Michel et al., 2005).

The effects of the *Ahl* mutation on vestibular structure and function are not well understood. The purpose of the present study was to evaluate whether the *Ahl* mutation, which when homozygous produces profound loss of hearing with age, has a similar effect on inner ear vestibular function. We characterized the effect of age and gender on macular function in B6 mice harboring the *Ahl* mutation and an inbred congenic strain, B6.CAST-*Cdh23*$^{Ahl+/Kjn}$ (B6.CAST), that is genetically identical to the B6 strain except for a small region of Chromosome 10 inherited from the CAST/Ei strain that contains the *Ahl* locus (Johnson et al., 1997; Keithley et al., 2004). Thus, in the B6.CAST strain, the *Ahl* locus is replaced by the normal dominant wild-type allele that is resistant to ARHL. In the resistant B6.CAST strain, the progression of ARHL is delayed by about 3–6 months and attenuated compared to B6 (Keithley et al., 2004).

The main objectives of the present study were to address the following questions: (1) are there differences in age-related decline of auditory and gravity receptor function and are there gender differences in functional decline; and (2) will elimination of the *Cdh23*^{753A} mutation confer protection and modify the vestibular aging profile? To answer these questions, we measured VsEPs to assess macular function and auditory brainstem responses (ABRs) to characterize auditory function in female and male B6 and B6.CAST mice. Confocal microscopic images of the utricle were quantitatively compared to determine if any age-related functional change in gravity receptor function might be explained by structural changes, especially synaptic morphology of sensory neuroepithelium.

2. Methods

2.1. Animals

Breeder pairs for C57BL/6J (stock #000664) and B6.CAST-*Cdh23*$^{Ahl+/Kjn}$ (stock #002756) were purchased from the Jackson Laboratory (Bar Harbor, ME, USA) and colonies of both strains were established at East Carolina University. Both sexes of B6 (n = 158, ages 1.02–23.8 months) and B6.CAST (n = 109, ages 2.1–24.5 months) were studied. Table 1 indicates the number of animals measured for ABR and VsEPs. Animals were housed in standard polyurethane cages grouped by gender within a temperature-regulated (21 + 3/−4 °C) room under routine 12-hour light and/or dark cycle with access to food and water ad libitum. Animal care and all procedures were approved by the Institutional Animal Care and Use Committee and met NIH guidelines for the care and use of laboratory animals.

Table 1
Number of data points for each threshold measure

Strain	8 kHz	16 kHz	32 kHz	41.2 kHz	VsEP
B6	75 females	75 females	73 female	74 female	68 females
	75 males	74 males	75 male	75 males	71 males
	150 total	149 total	148 total	149 total	139 total
B6.CAST	31 female	31 female	31 female	30 female	49 female
	42 male	40 male	38 male	38 male	55 male
	73 total	71 total	69 total	68 total	104 total

The table indicates the number of mice tested for each measure and includes cases where the measurement was performed but there was no measurable response (no response).
Key: VsEP, vestibular sensory evoked potentials.

Ambient noise levels in the animal housing area were monitored throughout the study to assure that noise levels were below those which might result in noise-induced hearing loss. The ambient sound exposure levels for animals in laboratory housing areas were estimated using a data logging dosimeter. Forty-eight noise samples, each comprised of 16-hour periods were taken on randomly chosen days over a 5-month period. Logged data were discretized into mean sound pressure level (SPL) for each 10-minute period of recording. The logger had a sound floor of 65 dB SPL, so all levels below that were given a value of 65 dB SPL. The mean ambient level across all logs was 66.8 dB SPL. One 10-minute period registered 85 dB SPL and was the maximum level recorded. Six 10-minute periods were between 75 and 80 dB SPL. Thirty-three periods were between 70 and 75 dB SPL, whereas the remaining 4568 ten-minute periods were below 70 dB SPL.

2.2. Functional assessment of vestibular and auditory sensors

Animal preparation and functional testing for VsEPs and ABR followed procedures described by Mock et al. (2011) and Vijayakumar et al. (2015). Briefly, mice were anesthetized with a ketamine (18 mg/mL) and xylazine (2 mg/mL) solution (5–9 μL/g body weight injected intraperitoneally). Core body temperature was maintained at 37.0 ± 0.1 °C. Linear acceleration pulses, 2-ms duration, were presented to the cranium via a noninvasive spring clip that encircled the head anterior to the pinna and secured the head to a voltage-controlled mechanical shaker. Stimuli were presented along the naso-occipital axis at a rate of 17 pulses/sec. Stimulus amplitude ranged from +6 dB to −18 dB re: 1.0 g/ms (where 1 g = 9.8 m/s^2) adjusted in 3 dB steps. Stainless steel wire was placed subcutaneously at the nuchal crest to serve as the noninverting electrode. Needle electrodes were placed posterior to the left pinna and at the hip for inverting and ground electrodes, respectively. Traditional signal averaging was used to resolve responses in electrophysiological recordings. Ongoing electroencephalographic activity was amplified (200,000X), filtered (300–3000 Hz), and digitized (100-kHz sampling rate). Two hundred fifty-six primary responses were averaged for each VsEP response waveform. All responses were replicated. A broad band forward masker (50–50,000 Hz, 94 dB SPL) was presented during VsEP measurements to verify absence of cochlear responses.

For ABR measurements, pure tone burst stimuli were generated and controlled using National Instruments data acquisition system and custom software. Tone bursts at 8, 16, and 32 kHz, 41.2 kHz had 1.0-ms rise and fall times with 1.0-ms plateau (3-ms total duration). Stimuli for ABR testing were calibrated using a Bruel & Kjaar ¼ inch microphone and Nexus amplifier. Stimuli were calibrated in dB peak equivalent SPL (peSPL) and were presented via high frequency transducers (ED1 driver, EC1 speakers, Tucker–Davis Technologies) coupled at the left ear via a modified commercial ear tip (ER 10D-T03, Etymotic Research, Inc). Auditory stimuli were presented at a rate of 17 stimuli/s. ABR intensity series were collected by reducing the stimulus in 10-dB steps at higher stimulus levels and 5-dB steps closer to threshold. At the end of functional testing, mice were euthanized, and temporal bones were dissected for immunohistochemistry and confocal microscopy.