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Anatomic correlates of strial presbycusis in recombinant inbred mouse strains

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**ANATOMIC CORRELATES OF STRIAL
PRESBYCUSIS IN RECOMBINANT INBRED
MOUSE STRAINS**

by

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**A Capstone Project
submitted in partial fulfillment of the
requirements for the degree of:**

Doctor of Audiology

**Washington University School of Medicine
Program in Audiology and Communication Sciences**

May 20, 2011

Approved by:

**Kevin K. Ohlemiller, Ph.D., Capstone Project Advisor
Keiko Hirose, M.D., Second Reader**

Abstract: This project attempts to identify anatomic features that predict the range of the 'normal' endocochlear potential in young inbred mice. Cochlear lateral wall histologic metrics were compared in recombinant inbred (RI) mouse strains formed from BALB/c and C57BL/6 mice.

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Introduction

Age-related hearing loss (ARHL), also known as presbycusis, is one of the most common diseases affecting the aged. According to the National Institute on Deafness and Other Communication Disorders, one in three people above age 60 and half of those over 85 have hearing loss (2001). This condition continues to proliferate as the population grows and medical advances provide a longer lifespan. Treatments such as hearing aids, hearing assistive technology, and cochlear implants have been very successful in improving the quality of life for those with presbycusis, but identification and protection of the anatomic structures that underlie this pathology would be even more significant.

Forms of presbycusis

In a pioneering study of 21 presbycusis human temporal bones, Schuknecht and Gacek (1993) identified three major forms of presbycusis that are most commonly accepted today. These include sensory (associated with initial pathology of the organ of Corti), neural (primary loss of neurons, while hair cells survive), and strial (degeneration of stria vascularis and subsequent reduction of the endocochlear potential (EP)). Each of these forms was posited to have its own set of environmental and genetic causes, and to give rise to a particular audiometric shape. Strial presbycusis has been claimed by some to represent the major and ‘purest’ form (Gates and Mills, 2005), being less easily confused with noise injury, and potentially arising from true ‘aging-like’ processes. The foundations of strial presbycusis have relied largely on a small set of temporal bones, and until recently, only a single clear animal model, namely the Mongolian gerbil (Schulte and Schmiedt, 1992; Gratton and Schulte, 1995; Spicer and Schulte, 2005). The recent appearance of suitable mouse models (Ohlemiller, 2009) has offered new

insights regarding when and how strial presbycusis arises. One of these models forms the basis of the present study.

Strial architecture and the generation of the EP

The stria vascularis is a three-layered, highly-vascularized epithelium found on the lateral cochlear wall (Fig.1). It is the central structure involved in normal endolymph composition and EP generation (Wangemann, 2002).

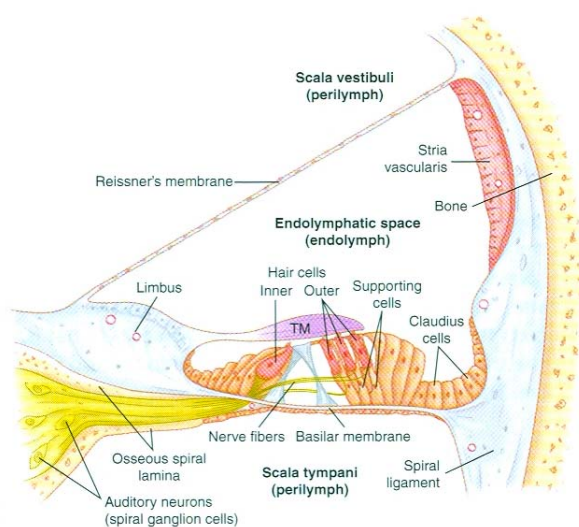


Figure 1: Schematic view of a portion of the membranous cochlea. Reprinted with permission from Bohne, 2008.

The ~90-100 mV EP is the driving force of sensory conduction in the cochlea, and requires a carefully regulated supply of K^+ —by far the dominant ion in endolymph— as its current carrier. The essential strial elements for EP generation are the *Kcnj10* channel, located in the intermediate cells, and the Na^+/K^+ -ATPase and $Na^+/K^+/Cl^-$ co-transporter in marginal cells. The DC electric potential that is the EP is created by positive charge concentration in endolymph in relation to perilymph (Salt et al., 1987; Takeuchi et al., 2000; Wangemann, 2006; Ohlemiller, 2009).

Essentially all individuals will show some degree of stria degeneration with age, manifested in the form of stria thinning, capillary loss, and loss of all three major cell types: marginal cells, intermediate cells, and basal cells (Fig. 2).



Figure 2: Radial section of stria vascularis and spiral ligament. Reprinted with permission from Bohne, 2008.

The stria, however, appears to possess an immense amount of extra functional capacity, so that only some people will exhibit EP decline and resulting hearing loss. If the stria degenerates to some threshold level, generation of the EP is impaired, resulting in increased hearing thresholds (Pauler et al., 1988). Strial presbycusis can manifest itself any time during the third to sixth decade of life. Purportedly, it is characterized by a flat or slightly sloping audiometric pattern which progresses over time (Schuknecht, 1964; Pauler, Schuknecht, and White, 1988; Schuknecht and Gacek, 1993).

Strial Presbycusis in the Mongolian Gerbil

While human temporal bones have been studied in the past and provided much insight into the pathology of presbycusis, EP has never been measured in humans. For this reason, researchers have turned to animal models in order to correlate declining EP with specific

anatomic features. Some of the first animals to be studied in this regard were Mongolian gerbils. In 1992, Schulte and Schmiedt chose to study gerbils because of their well characterized auditory system, short life span, and audibility curve that closely parallels that of humans. They found that striaal atrophy increases in prevalence and severity with age, supporting the idea that loss of striaal function leads to a lowering of the EP, subsequently elevating pure tone thresholds. Also of note was the tendency of striaal atrophy to be more prevalent in the apex earlier in life (Schulte and Schmiedt, 1992). Several years later Gratton and Schulte asserted that EP decline is associated with capillary loss, and that atrophy of the stria is secondary to the lack of vasculature (1995). More recently, however, studies have shown that degeneration begins in the marginal cells, with capillary loss being secondary. Specifically, atrophy begins at the secondary processes of the marginal cells, followed by the primary processes (Spicer and Schulte, 2005).

Strial Presbycusis in Mice

In neither humans nor animals is striaal presbycusis likely to represent a homogeneous process. But with forever limited human temporal bone samples, virtually no functional information in humans, and only a single animal model, there were few resources for uncovering multiple etiologies. In 2006, Ohlemiller, Lett, and Gagnon sought to establish the first naturally occurring mouse model of striaal presbycusis. In this study they compared cochlear lateral wall metrics in BALB/cJ (BALB) mice to those in C57BL/6J (B6) mice. The EP in the BALB mice decreased over their lifespan, concurrent with loss of marginal cells and thinning of the spiral ligament. B6 mice maintained a stable EP while retaining more marginal cells and spiral ligament thickness. Interestingly, the early 'normal' EP in BALBs was also slightly lower than in B6, as were initial marginal cell density and ligament thickness (Fig. 4). In this study,

marginal cell density and ligament thickness were found to be the most predictive of EP in the two strains (Fig. 3).

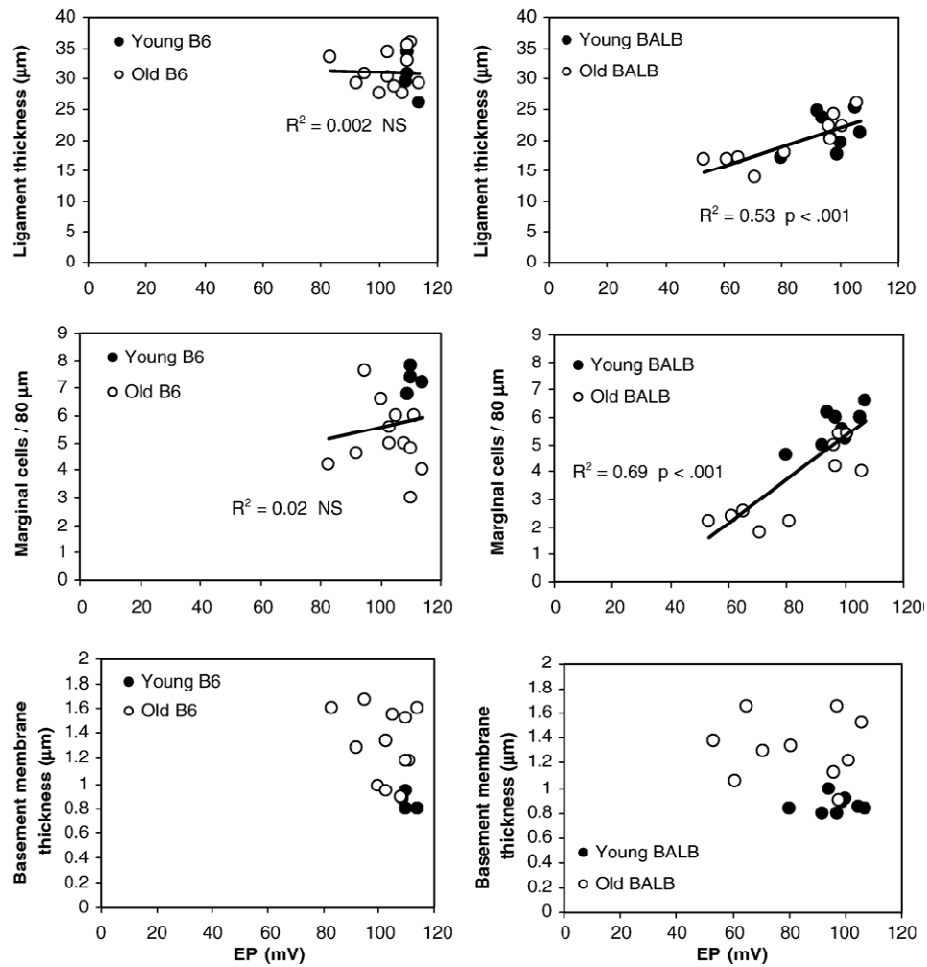


Figure 3 – Relation between basal cochlear turn EP and spiral ligament thickness (top row), strial marginal cell density (middle row), and strial capillary basement membrane thickness (bottom row) in female B6 and BALB/c mice. Each plot compares young (<3 months) and old (>19 months) within strain. Data for each animal are means from examination of upper basal turn in 5 sections. Reprinted with permission from Ohlemiller, 2006.

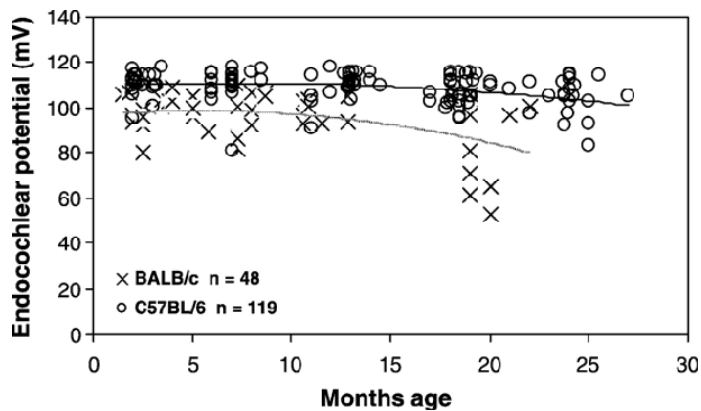


Figure 4 – Comparison of cochlear basal turn EP in B6 and BALB/c mice for ages up to 26 months. BALBs show EP reduction in some animals by 19 months (female only tested for ages >13 months). B6 (both genders at all ages) show no significant EP reduction. Initial ‘normal’ EPs in BALBs are ~10 mV lower than in B6. Reprinted with permission from Ohlemiller, 2006.

Surprisingly, changes in microvasculature such as capillary loss and capillary basement membrane thickening did not appear to be factors (Ohlemiller et al., 2006; Ohlemiller, 2009). These findings agree with earlier work identifying marginal cells as the first structures to degenerate in strial presbycusis in humans and animals (Schuknecht, 1974; Spicer and Schulte, 2005). However, they also added a previously unreported relation between strial marginal cell density and spiral ligament thickness. Subsequent studies supported this relation in another mouse model, the aging B6 albino mouse (Ohlemiller et al., 2009). The mouse results suggested a causal link between mildly reduced marginal cell numbers, a thin spiral ligament, and a reduced EP early in life. They further suggested that these are risk factors for strial presbycusis later in life. Finally, if these features co-occur only in certain individuals, they may be genetically linked, and such individuals may carry 'strial presbycusis genes'.

Pursuing the genetics of EP decline using Recombinant Inbred Mouse Strains

The stark differences in EP and cochlear lateral wall attributes between BALB and B6 mice suggest that these strains carry different alleles at one or more responsible gene loci. When two mouse strains usefully differ, this presents an opportunity to test the genetic bases of those differences. Typically, this is done by crossing the two strains and examining the phenotype of F1 hybrids, then F2 or N2 mice. In the case of BALB and B6 mice, however, there exists an alternative, in the form of 13 recombinant inbred (RI) mouse strains formed from these. RI strains are created by crossing two strains of mice, then creating sublines through sib crosses. This process essentially fixes a random mix of BALB and B6 genomes. Each of 13 established CxB RI strains (CxB1-CxB13) possess unique genomes and their related phenotypes. If a small number of genes are responsible for EP and lateral wall differences between BALB and B6 mice,

then some of the 13 RI strains will fix those same genes. If, say, the ‘BALB EP phenotype’ is due to one gene that has few modifiers, then roughly half of the CxB strains should be BALB-like. If, however, the phenotype is associated with two genes, then only a quarter of the RI strains should be BALB-like. Pilot experiments in the Ohlemiller laboratory have established an ordering of mean ‘normal’ EPs for 12 of the 13 RI strains. It was found that there is no clear delineation between BALB-like and B6-like RI strains. Nevertheless, ordering the 12 RI strains by mean EP yielded strains with significantly different EPs at each end of the continuum (Fig. 5).

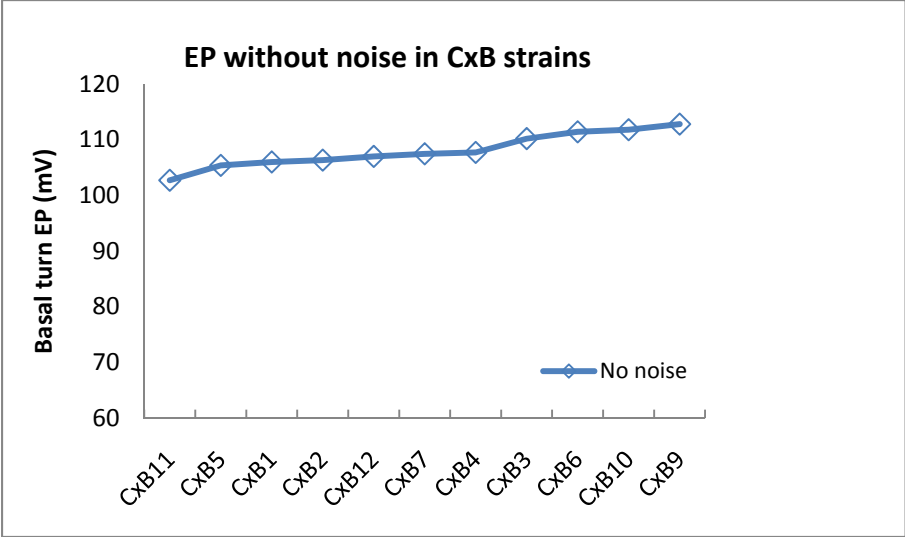


Figure 5: Young normal EP values in CxB strains. Strains have been ordered by mean EP (Ohlemiller, 2010, unpublished).

If the strains at opposite ends of the continuum show significantly different marginal cell densities and thicknesses of spiral ligament, it will argue that those strains fix the same alleles of (probably multiple) genes as to BALB and B6 mice. This expectation forms the foundation of this study.

Methods

All procedures were approved by the Washington University Institutional Animal Care and Use Committee. The sample was comprised of 4 recombinant inbred strains with EPs at the extremes of the distribution. Thus the two strains with the highest EP early in life (CxB9 and

CxB10) were compared with the two with the lowest EP early in life (CxB5 and CxB11) (Fig. 5). Mice included both genders and ranged from 2-4 months in age. Plastic-embedded left cochleae from 6 mice of each strain were sectioned in the mid-modiolar plane. Fifty 4 μ m sections were taken from each animal, spanning 200 μ m through the mid-modiolar core.

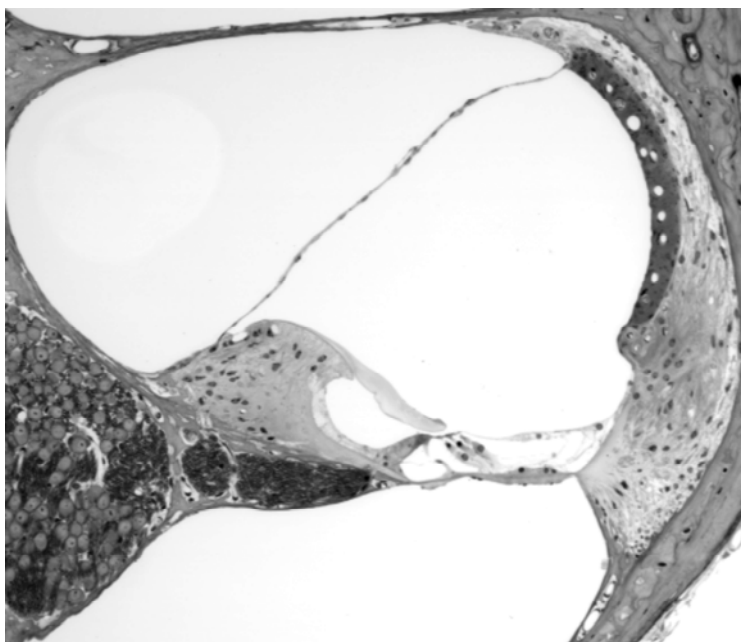


Figure 6: Lower base section of CxB11 mouse from the current study.

Ten sections from each mouse were analyzed at 3 locations: lower base, upper base, and lower apex. Metrics included marginal cell density, strial thickness, spiral ligament thickness, and capillary density. Marginal cells were counted in a linear 80 μ m section of the mid-point of the stria. Strial thickness was measured orthogonal to the midpoint, and ligament thickness was measured along a co-linear axis. Capillaries were counted along the entire profile of each stria. Metrics were obtained using a Nikon Optiphot™ light microscope with a 100x oil objective and a calibrated grid ocular.

Ten estimates for each animal were averaged to obtain overall means for each of the four histological metrics. Statistical analyses included a two way analysis of variance (ANOVA)

(metric x strain, cochlear location), followed by pairwise Bonferroni multiple comparisons tests (SIGMASTAT). Tests yielding a p-value less than 0.05 were considered to be significant.

Results

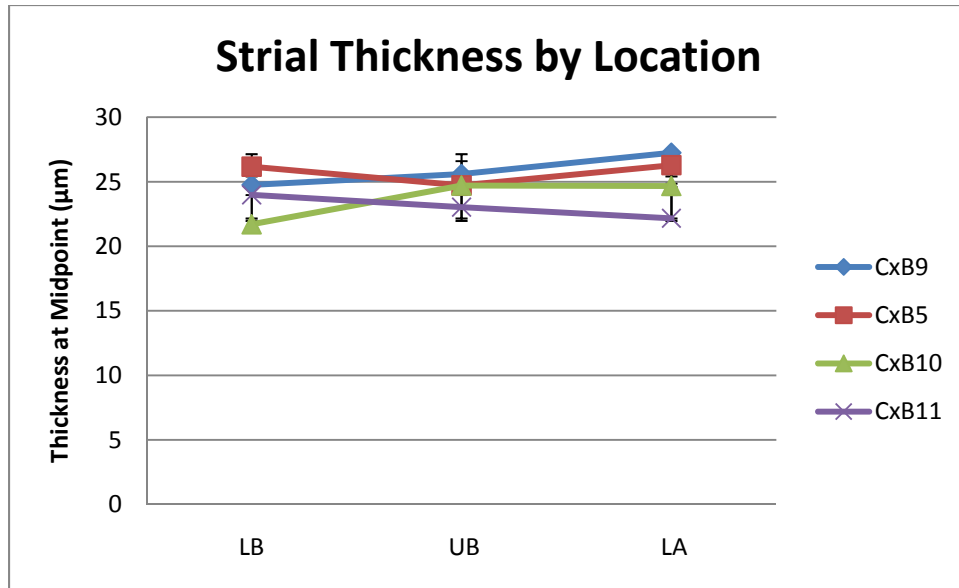


Figure 7: Average strial thickness measures taken at 3 locations: lower base, upper base, and lower apex.

As shown in Figure 7, strial thickness was similar at all three locations in all four strains.

There appeared no significant differences by strain.

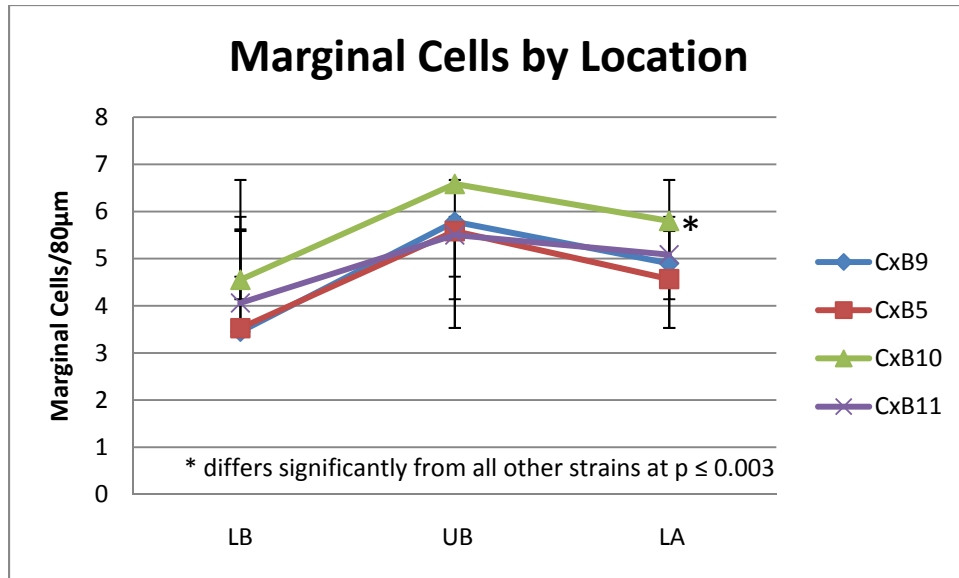


Figure 8: Average marginal cell densities measured at 3 locations: lower base, upper base, and lower apex. Significant difference denoted by [*].

CxB9, CxB5, and CxB11 strains all have similar marginal cell densities at each location.

However, CxB10 mice have overall significantly greater marginal cell density (Fig. 8).

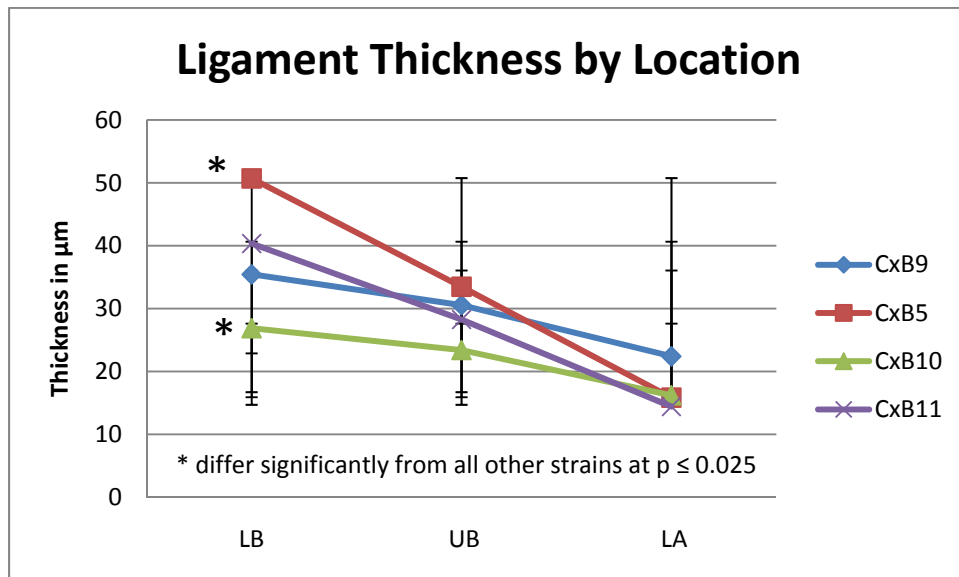


Figure 9: Average ligament thickness at three locations: lower base, upper base, and lower apex. Significant differences denoted by [*].

Not surprisingly, the spiral ligament becomes progressively thinner from the lower base to the lower apex. Overall, CxB10 mice showed a significantly thinner ligament than all other strains examined. By contrast, CxB5 mice—a ‘low EP’ strain—showed a significantly thicker ligament than all other strains. Differences were most pronounced in the lower base (Fig. 9).

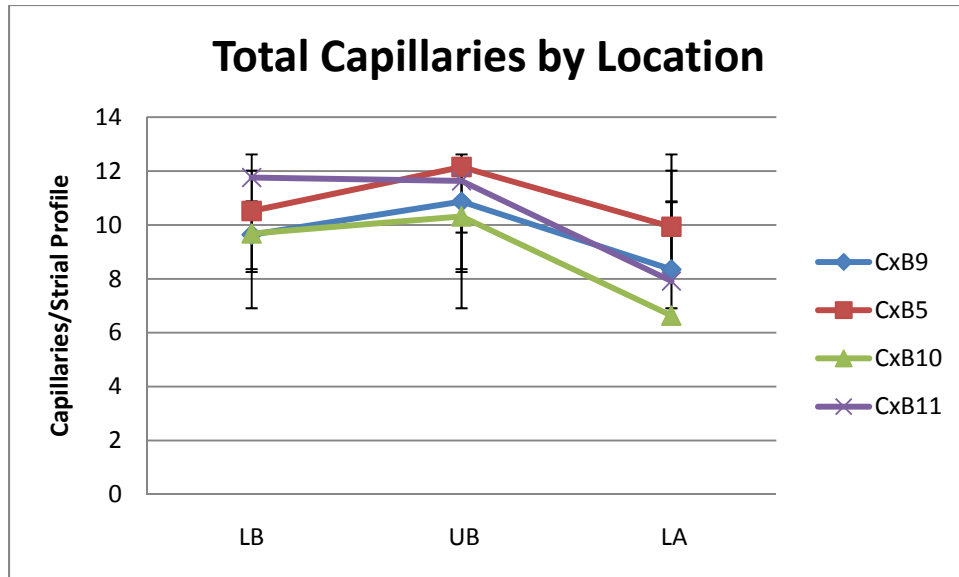


Figure 9: Average capillary densities measured at 3 locations: lower base, upper base, and lower apex.

Capillary density is similar for all strains at all locations, with fewer capillaries overall at the lower apex. There appeared no significant differences by strain (Fig. 10).

Discussion

In 2006, Ohlemiller and colleagues found marginal cell density and ligament thickness to be the most predictive of the EP in BALB mice, and the best predictors of EP differences between and BALB and B6 mice. When plotting basal cochlear turn EP against spiral ligament thickness and marginal cell density, a relation between strial marginal cell density and spiral

ligament thickness emerged (Fig. 3). These findings suggested a causal link between mildly reduced marginal cell numbers, a thin spiral ligament, and a reduced EP early in life. It was also postulated that these could be genetically linked risk factors for strial presbycusis in adulthood. The present study suggests that ligament thickness and marginal cell density need not be related to one another. This is perhaps most evident when considering the data from CxB10 mice. These mice were found to have significantly greater marginal cell density than the remaining three strains. If there was, in fact, a relation between marginal cell density and ligament thickness, one would expect the CxB10 mice to also have the thickest ligament measures. Instead, the current data indicate that this strain has the thinnest spiral ligament. Overall, there appeared no tendency for mice from the two strains with the highest EP to have more marginal cells, or a thicker ligament. Likewise, there appeared no tendency for mice from the two strains having the lowest EP to possess fewer marginal cells or a thinner ligament.

The published observations in BALB mice (Ohlemiller et al., 2006) suggest that these mice segregate alleles that jointly promote a smaller marginal cell population and a thinner spiral ligament early in life. While it remained unclear whether and how these interact, both seem tied to a somewhat lower EP early in life in BALBs. If these traits are tied to the same gene, then RI strains showing a BALB-like EP should also show BALB-like marginal cell density and ligament thickness. Conversely, if these traits are tied to different genes, then the two traits should prove separable across the RI strains. The present data support the latter scenario, suggesting that multiple genes underlie the BALB lateral wall phenotype. Even if that is the case, one still might have expected EP to follow *one* of these traits, most likely marginal cell density. However, the present data indicate that, when marginal cell density and ligament thickness are separated, the EP follows *neither* reliably. It may be that both of these traits (and

their underlying alleles) must coincide to exert a strong effect on the average EP. The nature of such interactions remains to be explored.

Clinical Implications

Practical and ethical limitations to the study of presbycusis in humans make animal studies essential. It has been posited that BALB/c mice harbor alleles at one or more genes that promote EP decline late in life. Early modest EP reduction in these mice, and its anatomic correlates, have been proposed to constitute early indicators of the later EP decline. If so, then the genes responsible in BALBs may constitute ‘pro-strial presbycusis’ genes. Whether any of the RI strains exhibit late-life EP decline has not been determined. If there exists a link between marginal cell density, spiral ligament thickness and late-life EP decline, the present findings suggest that BALBs harbor multiple such genes, and further, that these must coincide for EP decline to manifest. Whatever these genes are, they may exert similar effects in humans.

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