# ANATOMIC AND GENETIC CORRELATES OF THE ENDOCOCHLEAR POTENTIAL IN RECOMBINANT INBRED MICE

Capstone Project

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By

ANNA L. KIENER

*****

The Ohio State University
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Capstone Committee:

ERIC C. BIELEFELD, Ph.D., Advisor

CHRISTINA M. ROUP, Ph.D.

GAIL M. WHITELAW, Ph.D.

ERIC W. HEALY, Ph.D.

Approved by

_____

Advisor

# ABSTRACT

Since the endocochlear potential (EP) cannot be measured in humans, mouse models offer a useful way to investigate the pathophysiology of strial presbycusis and to potentially identify responsible genes. Two inbred strains of mice (C57BL/6J and BALB/cJ) have been shown to differ in terms of the EP and strial anatomical correlates. In contrast to C57BL/6J (B6) mice, BALB/cJ (BALB) mice have a lower EP, which coincides with a decreased number of marginal cells and a thinner spiral ligament in BALBs. To further investigate this relationship, and estimate the number of gene(s) responsible for the lower EP in BALBs, 11 recombinant inbred (RI) strains created from crosses between B6 and BALB mice were examined. Cochlear morphometric data gathered for the RI strains suggest that the responsible genes most prominently influence marginal cell density in the stria vascularis. Analysis of EP data using WebQTL online mapping utility revealed a suggestive linkage to a ~7Mb segment of Chromosome 12 (provisionally named *Nvep1*) and a suggestive linkage to Chromosome 15 (*Nvep2*). Analysis of marginal cell data using WebQTL revealed a suggestive linkage to the same ~7Mb segment of Chromosome 12 (*Nvep1*). Since *Nvep1* maps to same general location as does marginal cell density, this supports the suggestion that subtle differences in marginal cell density (or a related property) in part determine the EP. Since marginal cell density corresponds to both the

'low normal' EP in young BALBs as well as age-associated EP decline in BALBs, the *Nvep1* region may encompass a 'strial presbycusis' predisposing gene.

# ACKNOWLEDGMENTS

I would like to thank my mentor, Dr. Kevin K. Ohlemiller, for allowing me to contribute to this project and for his encouragement and patience. I would also like to thank my capstone committee, and my advisor, Dr. Eric C. Bielefeld, for his guidance and support.

## VITA

1/31/84. . . . . Born – CARBONDALE, IL

6/10/2006. . . . . B.A., SPANISH
The Ohio State University.

2009 – 2010. . . . . TEACHING ASST.
The Ohio State University.

2010 – 2011. . . . . RESEARCH ASST.
The Ohio State University.

## FIELDS OF STUDY

Major Field: Audiology

TABLE OF CONTENTS

LIST OF FIGURES

# LIST OF ABBREVIATIONS

| | |
|---|---|
| ARHL | age-related hearing loss |
| BALB | BALB/cJ |
| B6 | C57BL/6J |
| EP | endocochlear potential |
| IHC | inner hair cell |
| K+ | potassium |
| LOD | log of odds |
| Mb | megabase |
| mV | millivolt(s) |
| OHC | outer hair cell |
| QTL | quantitative trait locus |
| RI | recombinant inbred |
| SDP | strain distribution pattern |

# CHAPTER 1

# INTRODUCTION

Age-related hearing loss (ARHL), or presbycusis, is caused by the gradual degeneration of the auditory system over time, and is one of the most common chronic conditions affecting the aging population (Moscicki, Elkins, Baum, & McNamara, 1985; Cruickshanks, Wiley, Tweed, Klein, B., Klein R., & Mares-Perlman et al., 1998). According to the National Institute on Deafness and Other Communication Disorders (2001), 30-35% of adults over 65 have a hearing loss, with the prevalence increasing to 40-50% for individuals 75 and older. The number of individuals affected by this condition continues to grow with increases in lifespan and population growth. In the United States specifically, it is estimated that there will be 71 million adults over the age of 65 by 2030 (Center for Disease Control, 2007). A large proportion of this growing aging population will have ARHL, increasing the need for prevention and treatments that address communication difficulties posed by presbycusis.

Presbycusis may cause significant communication difficulties and a reduced quality of life, which can be partially managed by hearing aids, cochlear implants and assistive listening technology (Chisolm, Johnson, Danhauer, Portz, Abrams, & Lesner et al., 2007; Maillet, Tyler, & Jordan, 1995). It is well-documented that hearing loss is

associated with psychosocial dysfunction, fatigue, depression, and withdrawal from social situations (Heine & Browning, 2002; Mulrow, Aguilar, Endicott, Tuley, Velez, Charlip et al., 1990; Kochkin, 2007). Hearing aids and other assistive devices can ameliorate communication difficulties associated with presbycusis (Mulrow et al., 1990; Chisolm et al., 2007). Despite the fact that hearing aids are capable of improving communication ability and quality of life for those with hearing loss, only about 20% of individuals who could benefit from hearing aids use them (Kochkin, 2007). Reluctance to use hearing aids is commonly based on factors such as cost, a perceived lack of benefit, and perceived stigma associated with the use of assistive devices (Kochkin, 1993). The fact that hearing aids are only used by a small fraction of those who could benefit from them increases the urgency of finding alternative therapies to prevent or treat presbycusis.

Although hearing aids and other assistive technology can improve the quality of life for individuals with presbycusis, there is currently no technology that can match the extraordinary processing capability of the normal auditory system and restore natural hearing. It is not surprising that assistive technology does not completely correct hearing difficulties experienced by those with presbycusis, considering the complexity of the auditory system. ARHL typically affects the sensorineural processing of the auditory system that is responsible for transducing acoustic input into complex electrical impulses that are transmitted to the auditory cortex in the temporal lobe of the brain (Musiek & Baran, 2007). The healthy cochlea contains approximately 15,000 sensory hair cells which are responsible for processing incoming sound and transducing the information into electrical impulses. Presbycusis can result from the degeneration of both the sensory

and supporting cells of the cochlea, resulting in a loss of the filtering and transmission of incoming sound (Chisolm, Willott, & Lister, 2003). The most advanced hearing aid technology is not able to replace the exquisitely complex processing of the normal sensorineural system. While researchers interested in hearing aid technology continue to create more sophisticated devices to treat hearing loss, the issue of presbycusis is also being addressed from the vantage point of research in basic hearing science. A better understanding of the pathology underlying presbycusis may offer ways to treat or prevent the condition altogether. Potential treatments or preventative measures could come in the form of pharmacological interventions or even gene therapy if the genetic determinants of presbycusis are uncovered.

## Types of Presbycusis

Presbycusis refers to hearing loss due to any age-related degeneration of the auditory system, and it is generally accepted that there are several distinct forms of presbycusis caused by separate pathologies. The most widely accepted framework of presbycusis was described by Schuknecht in 1964 and separates ARHL into three main forms: sensory, neural, and strial presbycusis. These classifications were later confirmed by Schuknecht's histologic analysis of 21 human temporal bones, which demonstrated that individuals with ARHL typically exhibited degeneration of one cochlear cell type (sensory, neural, or strial) that outpaced other peripheral deterioration (Schuknecht & Gacek, 1993). Although this organizational scheme is generally supported by animal models (Ohlemiller, 2006), there is currently no consensus regarding how many separate types of ARHL exist or which clinical findings characterize each pathology.

*Sensory Presbycusis*

Sensory presbycusis is thought to be caused by a degeneration of the sensory and supporting cells within the organ of Corti (Schuknecht, 1964). The sensory mechanism of the cochlea is comprised of approximately 3,500 outer hair cells (OHCs) and 12,000 inner hair cells (IHCs) which are responsible for processing and transducing incoming sound signals into electrochemical impulses that can be conducted to the auditory cortex via the 8$^{th}$ nerve (Musiek et al., 2007). The degeneration of the sensory structures typically begins with the OHCs in the basal portion of the cochlea (Spongr, Flood, Frisina, & Salvi, 1997). The audiometric findings associated with sensory ARHL are thought to include a high frequency sensorineural hearing loss with relatively good word recognition scores (Schuknecht, 1974; Schuknecht et al., 1993). The fact that basal sensory structures, and OHCs in particular, are also susceptible to damage from noise-exposure and ototoxins makes sensory ARHL one of the most difficult forms of presbycusis to distinguish from injury-related pathology (Ohlemiller & Frisina, 2008)

*Neural Presbycusis*

Neural ARHL is characterized by a degeneration of spiral ganglion cells that outpaces the loss of sensory and supporting structures in the organ of Corti (Schuknecht, 1974). Neuronal loss tends to occur equally throughout the length of the cochlea, and involves the deterioration of the soma, axon, and dendrites of the cells (Chisolm et al., 2003). The clinical findings in cases of neuronal hearing loss usually involve a gradual or steeply sloping sensorineural loss, with word recognition that is disproportionately poor compared to pure tone thresholds (Schuknecht, 1974). Although this is considered to be the classic audiometric profile of neural presbycusis, neither pure tone thresholds

nor word recognition results offers a reliable representation of spiral ganglion cell integrity (Chisolm et al., 2003). It has been reported that up to half of all neural fibers may be lost with little change in hearing thresholds (Pauler, Schuknecht, & Thornton, 1986), and significant threshold shift may not occur until 90% of nerve fibers are lost (Ohlemiller et al., 2008).

*Strial Presbycusis*

The stria vascularis is a highly vascularized epithelium that lines the lateral wall of the cochlea and is responsible for maintaining the endocochlear potential (EP) needed to drive normal hair cell functioning (Musiek et al., 2007). Strial presbycusis is caused by a degeneration of the stria vasularis which is believed to lead to a reduction in the EP, resulting in a hearing loss (Chisolm et al., 2003). The strial atrophy typically occurs in the middle and apical cochlear turns and involves the deterioration of several strial cell types (Ohlemiller et al., 2008; Chisolm et al., 2003). It has been posited that strial ARHL is the 'purest' form of presbycusis since the degeneration is distinct from pathology that may occur due to noise or other cochlear injury (Gratton & Schulte, 1995). Strial presbycusis is characterized by a flat or slightly sloping sensorineural hearing loss with well-preserved speech recognition ability (Schuknecht, 1974). The onset of strial ARHL typically occurs in the third to sixth decade of life (Schucknecht, 1964; Pauler, Schuknecht & White, 1988; Schuknecht et al., 1993). Schuknecht reported that strial ARHL appeared to have a highly genetic basis, which is supported by ARHL inheritance studies (Gates, Couropmitree, & Myers, 1999). The EP has not been measured in humans, and until recently, the most widely accepted animal model of strial presbycusis was the Mongolian gerbil (Schulte & Schmiedt, 1992; Gratton et al., 1995; Spicer &

Schulte, 2005). The recent emergence of various mouse models that exhibit characteristics of strial ARHL offers an opportunity to further study its pathophysiology and potentially uncover responsible genes.

*Anatomy and Physiology of the Cochlear Lateral Wall*

The stria vascularis is a highly vascularized three-layered structure that runs from base to apex, supplying the cochlea with blood and maintaining the +80-90 mV EP needed to drive hair cell receptor potentials (Takeuchi, Ando, & Kakigi, 2000). Normal hair cell transduction takes place when mechanically-gated ion channels between stereocilia at the top of hair cells open to permit the influx of positively-charged endolymph. This has the effect of depolarizing the normally negatively-charged IHCs and OHCs. Depolarization causes electromotile action in OHCs and firing of auditory neurons at the base of IHCs, both essential for normal cochlear functioning.

The stria vascularis is affixed to the lateral aspect of the cochlear capsule by the spiral ligament, which is a connective tissue structure composed of various types (I-V) of fibrocyte cells (Santi & Tsuprun, 2001). Medially, the stria vascularis is contiguous with the endolypmhatic space of scala media (Musiek et al., 2007). The three layers of the stria vascularis are made up of three distinct cell types: basal cells which border the spiral ligament, intermediate cells, and marginal cells which form the boundary between the stria and the endolymph of scala media.

The stria vascularis is responsible for recycling K+, which is the principle cation that contributes to the high EP needed for normal hair cell functioning (Spicer et al., 2005). The EP is a direct current electrical potential that exists between the positively charged endolymph of scala media and the neutral perilymph of the scala vestibuli and

scala tympani (Takeuchi et al., 2000; Wangemann, 2006). Spicer et al. (2005) have described three lateral currents that recover K+ from the hair cells and perilymphatic spaces and cycle it back into the endolymph via the spiral ligament and stria vascularis. The first recycling route begins in the OHCs, which have used the K+ for depolarization, and continues transcellularly through the supporting cells of the organ of Corti to return the positively charged ions to the stria (Spicer & Schulte, 1993, 1994a, b, 1996). A second recycling current recovers K+ from scala tympani and scala vestibuli after it has been expelled from hair cells and returns it to the stria vascularis via the fibrocyte cells of the spiral ligament (Zidanic & Brownell, 1990). A third recycling route is believed to transmit K+ from scala media and scala tympani, returning it to the stria vascularis through the cells in the spiral ligament (Spicer, Smythe, & Schulte, 2003). Figure 1 illustrates the general pathways of K+ as it is recycled by the stria vascularis. Once K+ is returned to the stria vascularis, several important ion channels are involved in generating the EP. The *Kcnj10* channel moves K+ through the intermediate cells, and the $Na^+/K^+$-ATPase and $Na^+K^+/Cl^-$ co-transporter channels are responsible for moving K+ through the marginal cells (Wangemann, 2006; Crouch, Sakaguchi, Lytle, & Schulte, 1997).

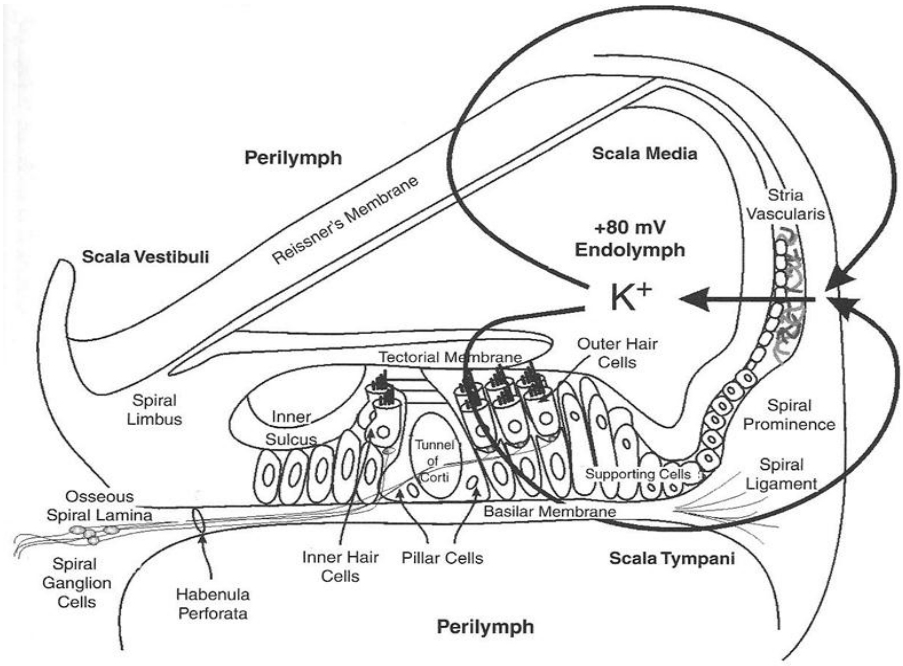


**Figure 1.** Lateral K+ recycling currents of the stria vascularis. (Used with permission)

The stria vascularis contains a dense network of capillaries and has a high metabolic demand due to its role in endolymph production. One possible etiology underlying strial presbycusis is microvascular dysfunction involving capillary loss and the thickening of capillary basement membranes (Johnsson & Hawkins, 1972; Gratton, Schmiedt, & Schulte, 1996). Although microvascular degeneration may be responsible for some cases of strial presbycusis, human temporal bone studies suggest that marginal cell pathology is the dominant cause of human strial presbycusis (Schuknecht, 1974). Applicable animal models, including the Mongolian gerbil and BALB/cJ mouse also exhibit an age-related decline in the number of marginal cells and an associated decrease in the EP (Schulte et al., 1992; Spicer et al., 2005; Ohlemiller Lett, & Gagnon, 2006). In

both humans and animals, 30 to 40% of the stria may degenerate before changes in hearing sensitivity occur (Pauler et al., 1988).

*Strial Presbycusis in the Mongolian Gerbil*

Although many animals exhibit some aspects of strial pathology, the Mongolian gerbil has been the most widely-used model of human strial presbycusis (Ohlemiller et al., 2008). It is thought that most of the ARHL that occurs in this species can be attributed to strial degeneration and a reduction in the EP (Schulte et al., 1992; Gratton et al., 1995; Spicer et al., 2005). Strial atrophy has been shown to begin in the secondary processes of marginal cells and spread to the primary processes which project from the apical cell bodies of marginal cells (Spicer et al., 2005). Because of the genetic heterogeneity of the Mongolian gerbil, this model is not well-suited for research attempting to isolate the genetic factors responsible for strial presbycusis (Ohlemiller et al., 2008). This drawback can be remedied through the application of mouse models of strial ARHL which facilitate genetic analysis through comparison with other readily-available inbred mouse strains.

*Advantages of Mouse Models*

Mice are an attractive model for ARHL for many reasons that include a genetic similarity to humans and the genetic homogeneity within strains. Information gathered about the mice genome can be applied to humans because mice and humans share a great number of similar genes or homologs; in fact, approximately 80% of all human and mouse genes are orthologous, meaning that they were inherited from a common ancestor (Griffiths, Wessler, Lewontin, & Carroll, 2008). Many mutually-inherited orthologous genes retain their original biologic function in the various species that inherit them

(Remm, Storm, & Sonhammer, 2001), which allows for direct genetic comparison between mice and humans. Mice models also offer practical advantages for hearing research such as their short life span, which reduces housing costs, and the fact that they have a great capacity for genetic standardization (Ohlemiller, 2006).

*Strial Presbycusis in Mice*

Until recently, the Mongolian gerbil was the only animal model known to exhibit the hallmark strial presbycusis features of strial degeneration starting with marginal cells and an associated reduction in the EP. Unlike the Mongolian gerbil, mice offer a multitude of inbred strains, which facilitates genetic analysis through the comparison of physical traits in strains with distinctly different genetic backgrounds (Ohlemiller et al., 2008). A 2006 study by Ohlemiller et al. identified BALB/cJ (BALB) mice as the first naturally-occurring mouse model of strial presbycusis for which a clear anatomic correlate could be found. Reduction in the EP of BALBs by 19 months of age corresponded to a decline in marginal cell numbers. In addition to the degeneration of marginal cells, a thinning of the spiral ligament was noted with age in BALB mice. A significant feature of the study was that the EP and cochlear lateral wall anatomical measures in BALB mice and C57BL/6J (B6) mice were compared side by side. In contrast to BALBs, the B6 mice maintained a stable EP throughout their lifespan that corresponded to a stable marginal cell population and a thicker spiral ligament. Figure 2 illustrates the fact that young B6 mice begin life with higher numbers of marginal cells and a thicker spiral ligament compared to age-matched BALB mice. In addition to the differences observed at a young age, B6 mice maintain a stable EP, a higher density of marginal cells, and a thicker spiral ligament throughout the lifespan compared to BALB

mice, which can be seen in Figure 3. Marginal cell density and spiral ligament thickness were significantly correlated with the EP in both young and old BALB mice. The existence of two usefully-differing mouse strains lends itself to investigation of the genetics underlying strial ARHL using recombinant inbred mouse strains, which is the basis of the present study.

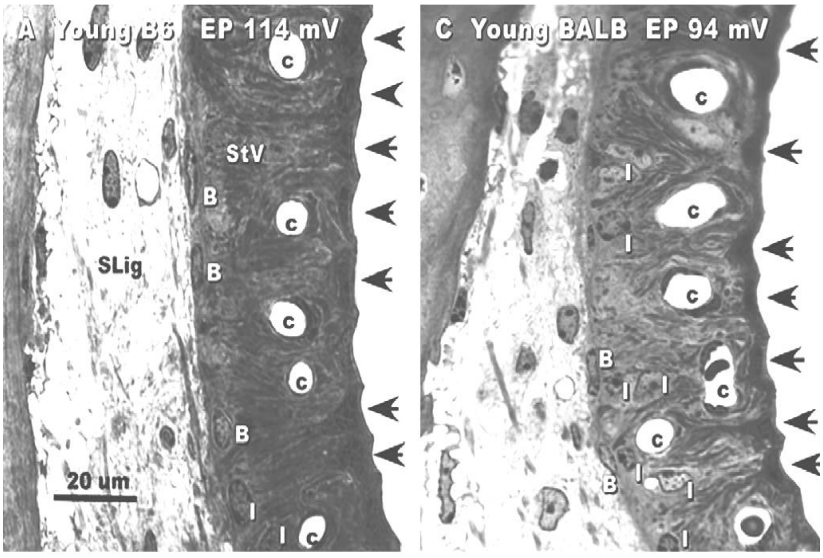


**Figure 2.** Typical upper basal lateral wall in BALB and B6 mice (Ohlemiller et al., 2006). Arrows indicate location of marginal cells.

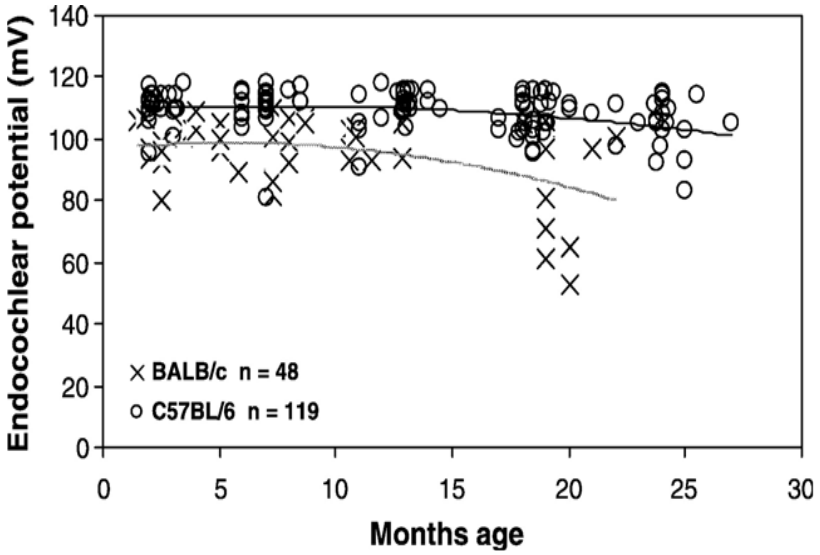


**Figure 3.** EP versus age for BALB and B6 mice. BALBs begin life with lower 'normal' EP and are more likely to exhibit EP decline (Ohlemiller, 2006).

*Pursuing the Genetics of Strial Presbycusis using Recombinant Inbred Strains*

The fact that BALB and B6 mouse strains usefully differ in terms of the EP and cochlear lateral wall attributes offers the possibility of using recombinant inbred (RI) mouse strains to investigate the genetic components responsible for these differences. Key to this innovation is the commercial development and availability of RI strains formed from BALB and B6. RI strains are created by crossing random offspring from two parent or progenitor strains, then intentionally generating sub-lines from a large number of F1 mating pairs. Each RI strain is rendered inbred through repeated sibling mating over 20 or more generations; this yields RI strains that are genetically different from one another, but essentially identical within any RI strain (Bailey, 1971). A set of RI strains derived from the same progenitor strains can be used to detect linkages

between a phenotype (i.e., endocochlear potential or lateral wall metrics) and previously mapped marker loci in the mouse genome (Neumann, 1992). Marker loci are molecularly distinct regions of chromosomes spread throughout the genome specifically chosen because they are polymorphic between the parent strains. Thus all the RI strains in a series will carry unique combinations of traits and adjacent markers that can be typed for one of the parent strains. Accordingly, the strain distribution pattern (SDP) for a trait can be expected to match the SDP of a known marker, permitting coarse mapping of the trait. Typically one may obtain a location known to derive from only one of the two parent strains that contains a chromosomal region that correlates with a trait of interest. Since the actual gene and gene product will frequently remain unknown, the region is referred to as quantitative trait locus (QTL), and is simply named for the trait itself (Alberts & Schughart, 2010). Growing sets of strain-dependent marker locations in established RI strains are continually updated in online repositories. A typical analysis entails phenotyping as many members of an RI series as possible, then entering the SDP into an online mapping utility such as WebQTL, which calculates the statistical phenotype-genotype association (Wang, Williams, & Manly, 2003). The identification of QTLs for the phenotype of interest allows researchers to pursue candidate genes that regulate the trait using traditional mapping and gene array methods.

*Present Study*

The fact that BALB and B6 mouse strains differ in terms of both the EP and cochlear lateral wall attributes suggests that they possess different alleles at one or more loci that control these features. Fortunately, there is a well-established RI strain set, the CxB series, which is formed from genetic crosses of BALB and B6 mice, facilitating

genetic linkage analysis of measureable traits. There are 13 members of the CxB series, denoted CxB1–CxB13. Each CxB strain carries a random assortment of the BALB and B6 genomes, but is homozygous at essentially all gene loci. The number of strains that exhibit either BALB or B6 characteristics can be used to estimate the minimum number of genes that regulate the EP and anatomical correlates (Bailey, 1971). For example, from simple Mendelian principles, if roughly half the RI strains possess a BALB-like phenotype, it would suggest that primarily one gene loci shapes the trait. If two genes are largely and equally responsible for the 'BALB EP' phenotype, approximately one quarter of the RI strains would be expected to inherit these genes and the associated phenotype. In pilot experiments conducted by the Ohlemiller laboratory in 2010, the EP was measured in 12 of 13 commercial CxB RI strains to establish the SDP for 'normal' EP across these RI strains. Figure 4 illustrates the distribution of mean EP for each of these 12 RI strains. Results revealed two strains (CxB5 & CxB11) that exhibited a clearly 'BALB-like' EP (≤ 105 mV), suggesting that two or more genes are responsible for the 'BALB EP' phenotype.

The present study was undertaken to further investigate the pathophysiology of strial presbycusis and to potentially identify responsible genes. The histopathology of strial ARHL was explored by comparing anatomical measures of stria vascularis and spiral ligament integrity to the EP measured across 11 members of the CxB series. Investigation of the potential gene(s) responsible for strial presbycusis was conducted by performing genetic linkage analysis using the cochlear lateral wall metrics and EP measurements.

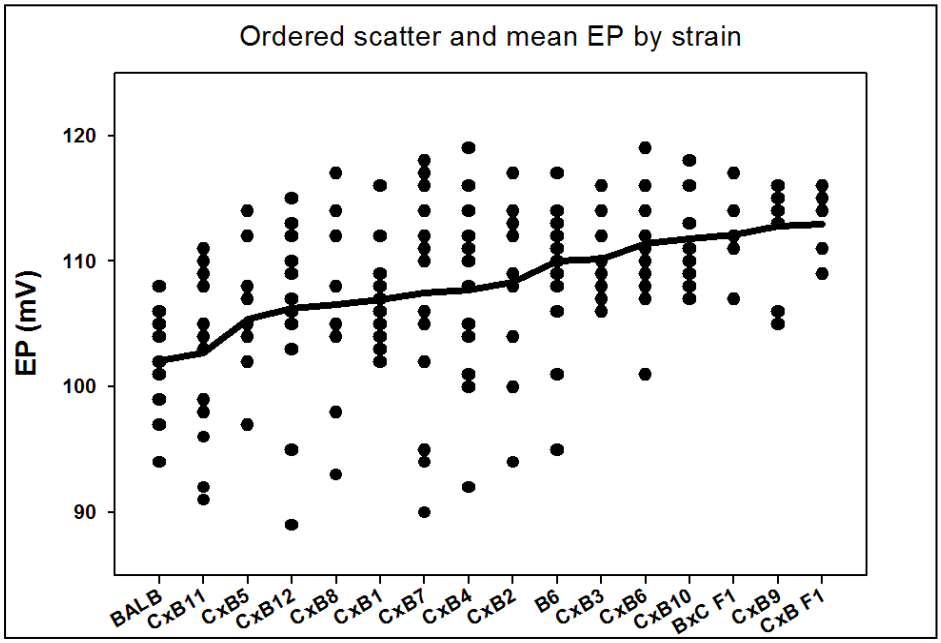


**Figure 4.** Mean EP ordered across 12 RI strains, reflecting effect of random combinations of B6 and BALB alleles on EP. (Ohlemiller, 2010, unpublished).

# CHAPTER 2

# METHODS

All procedures were approved by the Washington University Institutional Animal Care and Use Committee. The sample consisted of 11 of 13 RI strains (CxB1-CxB13) available from The Jackson Laboratory. The CxB13 strain was not analyzed due to significant middle ear pathology, and the CxB8 strain and F1s have not yet been analyzed. Mice included both genders and ranged from 2-4 months in age (typical life span of ~24 months). Young mice were examined in this study because the two progenitor strains (BALB and B6) are known to differ in terms of the EP and anatomical correlates even at a young age (Ohlemiller, 2006). The EP was measured in 6 mice from each strain. A fine drill was used to make a hole directly over scala media in the lower basal turn of left cochleae. Glass capillary pipettes (40-80 MX) filled with 0.15 MKCl were advanced into scala media until a stable positive potential was observed that did not change with increased electrode depth. Left cochleae were embedded in Epon plastic and sectioned in the mid-modiolar plane. Fifty 4 μm sections were obtained from each cochlea, spanning 200 μm through the mid-modiolar core. Ten sections were analyzed for each mouse. Analysis of cochlear metrics (strial thickness, marginal cell density, and spiral ligament thickness) was completed in three locations: lower base, upper base, and

lower apex. Marginal cells were counted in an 80 μm linear segment of the stria vascularis centered at the midpoint. Strial thickness was measured orthogonal to the midpoint. Spiral ligament thickness was measured on an axis co-linear with the strial midline. Cochlear metrics were obtained using a Nikon Optiphot™ light microscope with a 100x oil objective and a calibrated grid ocular.

Overall means for each cochlear metric were calculated by averaging the ten estimates for each animal. Linear correlations were calculated between EP and each strial metric (strial thickness, marginal cell density, and spiral ligament thickness) for B6, BALB, and 11 RI strains. Strial metrics were focused on the upper basal turn. For all correlations, a regression slope significantly different from zero was taken to denote a statistically significant difference between groups. Cochlear metric and EP measurements taken across the 11 RI strains yielded SDPs of each phenotype. Quantitative trait locus (QTL) mapping was performed by analyzing the SDPs for each phenotype (EP and cochlear metrics) using WebQTL to investigate statistical association between phenotype values and genotypes of marker loci.

# CHAPTER 3

# RESULTS

None of the strial metrics (strial thickness, marginal cell density, and spiral ligament thickness) were significantly correlated with the EP across the 11 RI strains (Fig. 5). Figure 5 shows correlations between the EP and each strial metric and illustrates the fact that the relationship between marginal cell density and the EP approached significance ($p < .06$).

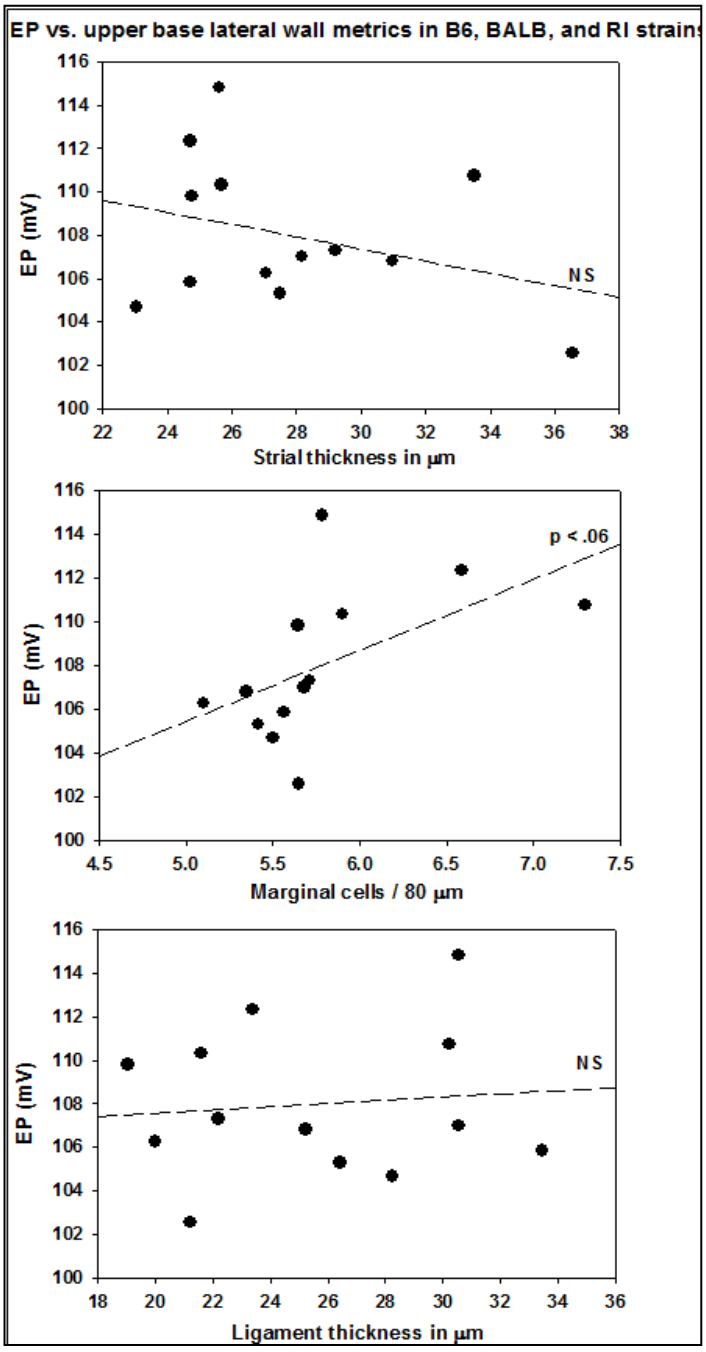


**Figure 5.** EP versus three strial metrics in 11 of 13 RI strains plus B6 and BALB. None were significantly correlated with EP, although relation to marginal cell density is suggested. F1s, CxB8, and CxB13 have not been analyzed.

Anaylsis of the SDP for EP using the online mapping utility WebQTL revealed a 'suggestive' association [log of odds (LOD) score > 4.12] with a segment of Chromosome 12. Figure 6 shows the likelihood of association between the EP and genetic loci on each mouse chromosome, with the 'suggestive' association on Chromosome 12. Since one or more genes within this region appear to be involved in regulating the EP, this QTL was provisionally named *Nvep1* to denote 'normal variation in EP'. A suggestive locus was also revealed on Chromosome 15, which was provisionally named *Nvep2*. Figure 7 shows an expanded view of the *Nvep1* region, which revealed that the locus encompasses a ~7Mb section of Chromosome 12.

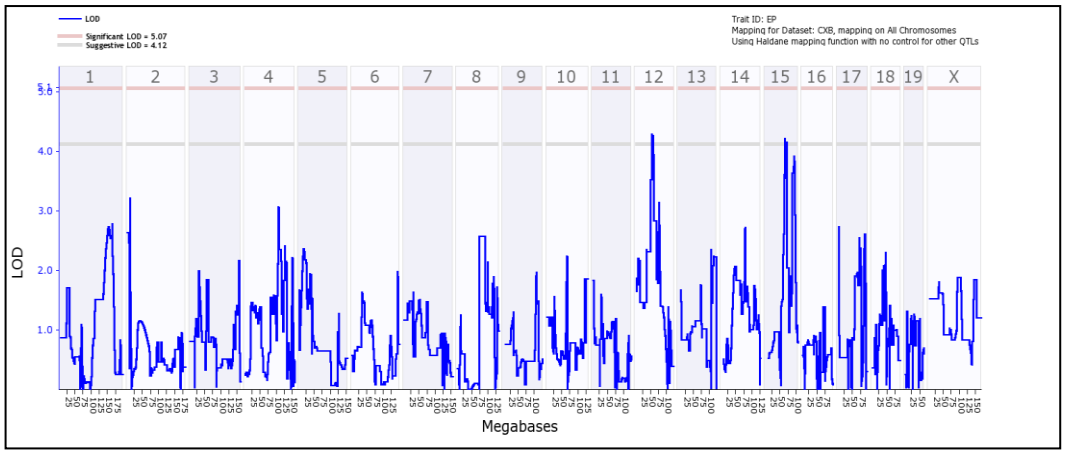


**Figure 6.** Analysis of EP data using WebQTL online mapping utility revealed 'suggestive' linkage to a segment of Chromosome 12 (provisionally named *Nvep1*) and suggestive locus on Chr. 15 (*Nvep2*).

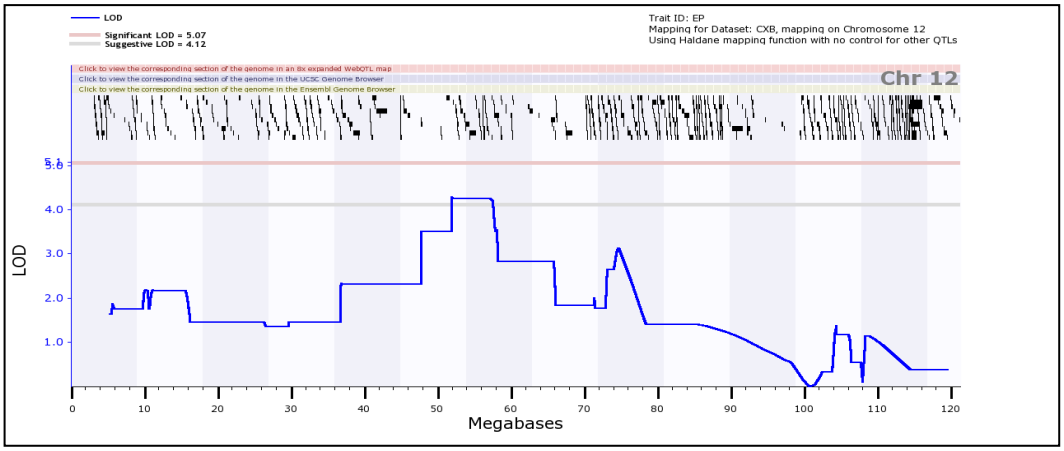


**Figure 7.** Expanded view of *Nvep1* region revealed a 'suggestive' log of odds (LOD) score (> 4.12) for EP and ~7Mb segment of Chromosome 12 .

Anaylsis of the SDP for marginal cell density also revealed a 'suggestive' association with the same region of Chromosome 12. Figure 8 illustrates the likelihood of association between the SDP for marginal cell density and each mouse chromosome, displaying the 'suggestive' association between marginal cell density and Chromosome 12. An expanded view of the *Nvep1* region, shown in Figure 9, revealed that the marginal cell SDP maps to the same ~7Mb segment of Chromosome 12 as did the SDP for EP.

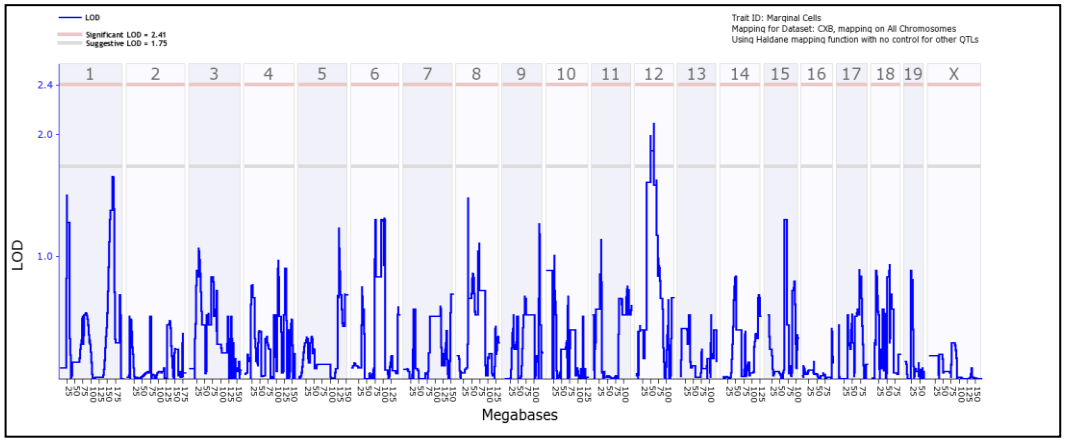


**Figure 8.** Analysis of marginal cell data using WebQTL revealed significant linkage to same ~7Mb segment of Chr. 12 (*Nvep1*).

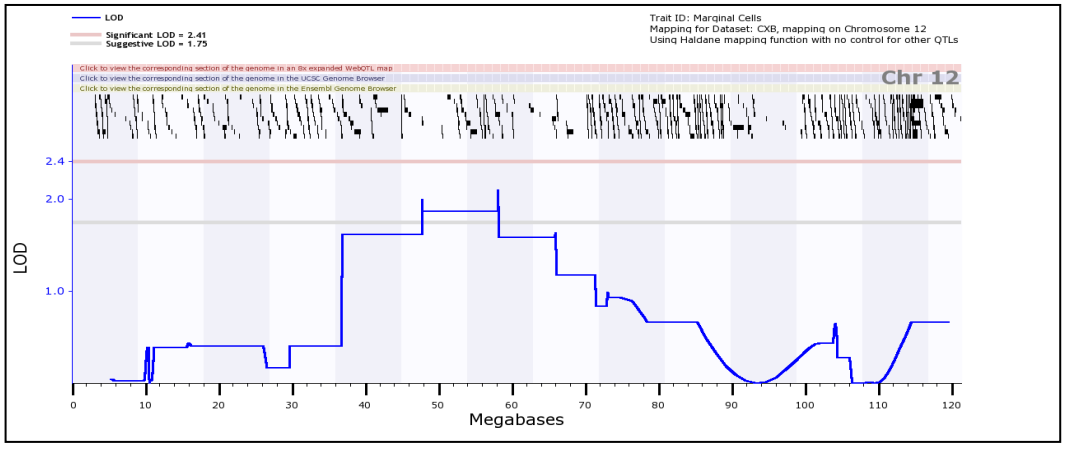


**Figure 9.** Expanded view reveals Chr. 12 'suggestive' log of odds (LOD) score (>1.75) for marginal cell data that aligns with the ~7mB segment of *Nvep1*.

# CHAPTER 4

# DISCUSSION

The overlapping QTLs associated with EP and marginal cell density offer support for the suggestion that subtle differences in marginal cell density (or a related property) in part determine the EP. Additional support comes from the fact that the correlation between the EP and marginal cell density approached significance ($p < .06$). This relation has been observed in human temporal bone and animal studies (Schuknecht, 1974; Ohlemiller et al., 2006; Spicer et al., 2005). The provisional QTLs, *Nvep 1* and *Nvep 2*, are the first loci that appear to modulate the EP within the normal range. Since marginal cell density corresponds to both the 'low normal' EP in young BALBs as well as age-associated EP decline in BALBs, the *Nvep1* region may encompass a 'strial presbycusis' predisposing gene.

The findings of this study are consistent with both human temporal bone and Mongolian gerbil studies. In his study of human temporal bones, Schuknecht (1974) observed that the degeneration of the stria appeared to begin with pathology of the marginal cells. Likewise, studies involving the Mongolian gerbil have revealed that the hearing loss observed in these animals is attributable to strial pathology, with cell death beginning with the marginal cells and involving other lateral wall cell types with time.

The findings of the present study, which revealed a suggestive linkage between a segment of Chromosome 12 (*Nvep 1*) with both marginal cell density and the EP offers further support for an association between marginal cell health and the EP. Although hearing threshold shift was not measured in mice from the present study, the anatomical and EP measurements agree well with the findings of previous studies (human temporal bones and Mongolian gerbil) which exhibited similar strial pathology and hearing loss. This agreement supports the fact that the BALB mouse is an appropriate model for strial presbycusis.

Potential limitations of the study include using cell counts from a single cochlear location to represent the integrity of the cochlea as a whole, and using only young mice. Future studies may involve examination of aging mice from the CxB sRI strains to determine whether the differences in EP and marginal cell density persist throughout the lifespan. The relationship between the cochlear lateral wall metrics (strial thickness, marginal cell density, and spiral ligament thickness) may become more obvious in older mice with more pronounced age-related strial pathology. Additionally, due in part to the relatively small set of CxB RI strains, the suggested QTLs must be confirmed using additional methods. The discovery of *Nvep1* and *Nvep2* paves the way for future studies aimed at determining the specific genes responsible for strial presbycusis. In particular, a future study may involve the creation of two congenic strains, each for one QTL that can be used to reduce the QTL interval for better isolation of candidate genes. Within the present interval for Nvep1 are 48 known genes, each of which can be evaluated using online data bases for known polymorphisms between BALB and B6 mice.

If *Nvep1* does indeed constitute a 'strial presbycusis predisposing' gene, its human homolog may operate in the same manner. It might thus be possible to identify clinical populations that would be considered at-risk for strial presbycusis. This might facilitate audiologic and/or medical treatment that is tailored to the underlying pathology of strial presbycusis. For example, the fact that individuals with strial presbycusis are thought to have well-preserved word recognition might influence an audiologist's prediction of hearing aid benefit or assist in the selection of a hearing aid fitting strategy. Strial presbycusis may also one day be corrected medically by an inner ear implantable device that restores the normal +80mV EP in the human cochlea. Such a device has been shown to be temporarily successful in improving thresholds in Mongolian gerbils with strial presbycusis by injecting positive current into scala media with an electrode (Schmiedt, 1993). In order to be a viable option for humans, this device faces the challenges of finding an electrode-insertion method that minimizes damage to the sensory structures of scala media and is able to maintain the DC voltage in the fluid-filled cochlea.

A more desirable alternative to a prosthetic device is a solution that repairs the cells involved in maintaining the EP. For example, strial presbycusis might one day be amenable to correction through gene therapy that would replace defective gene(s) in order to restore the function of cells involved in K+ recycling. Gene therapy may one day be used to introduce healthy genes into the bloodstream or respiratory system that would express themselves in the inner ear and correct strial presbycusis. While it is currently very difficult to separate the various forms of ARHL based on audiometric results, genetic testing offers the promise of an objective test that could provide

differential diagnosis and treatment options for strial ARHL. Regardless of whether gene therapy for strial ARHL becomes a reality, genetic hearing research using mouse models provides invaluable information about the pathophysiology of strial presbycusis, which will improve audiologic rehabilitation for the growing aging population in the future.

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