Gene Mutations: One of the Many Roads to Deafness

Shaun Davis*, Laura Hoholik*, Kushal Modi*, Lindsey Rockwell*
Department of Biology
Lake Forest College
Lake Forest, Illinois 60045

Summary

Deafness, a pathological condition causing partial or complete loss of hearing, affects nearly 70 million people worldwide. Research has linked nonsyndromic deafness to over 30 genes. In this review, implications of mutations on four of these genes will be discussed to understand how normal function is altered. While various mutations are linked to deafness, little is known about the mechanisms that lead to deafness. MyosinVI mutations hinder the motor protein's progressive movement, rendering the protein unable to stabilize stereocilia. Point mutations in PMCA2 gene in outer hair cells (OHCs) cause a defect in the calcium pump, leading to a change in calcium concentration in the cell and loss of hearing and imbalance. Mutations on the KCNQ4 gene in the OHCs cause K+ channel malfunction, ceasing the removal of K+ and inhibiting repolarization, thus leading to degeneration of the cell. Connexin 26 mutations lead to destruction and degradation of critical components within the inner ear, as well as problems with gap junction communication. These mutations cause autosomal recessive deafness. Currently, there is no cure for deafness, but development of treatments such as cochlear implants and other electrical systems can improve the quality of hearing. Identification and a deeper understanding of new genetic mutations can enhance treatments.

Introduction

Hearing disorders are the most frequently inherited sensory defect in humans, with nearly 70 million people across the globe suffering from a form of hearing loss (Wilson, 1985). Hearing disorders may be present at birth or occur gradually over a person's lifetime. Deafness, characterized as hearing loss, can be environmentally induced, syndromic, or nonsyndromic. Damage to the outer or middle ear by exterior sources can lead to environmentally induced deafness. Syndromic deafness occurs in conjunction with other abnormalities unrelated to audition. This form can be dominant, recessive, x-linked, or mitochondrial. In contrast, nonsyndromic deafness occurs without recognizable symptoms and can be x-linked, dominant, or recessive (Schrijver, 2004). This review focuses on autosomal dominant and recessive forms of nonsyndromic deafness.

Mutations in over 30 genes, many of which correspond to processes and development of the inner ear, have been related to nonsyndromic deafness. Mutations on genes associated with deafness cause a variety of defects in the processing of sound. Symptoms of deafness include hearing difficulties and ringing in the ears. However, in children, hearing impairment may be difficult to diagnose. Failure to respond to sounds, disinterest in music, and lack of verbalization are noticeable signs that can help lead to diagnosis (Piatto, 2005). Children should be screened for hearing loss so that deafness can be detected as early as possible.

Hearing loss treatment for children and adults has evolved over the years. Historically, treatments used were devoid of any biological bases such as inserting a stick into the ear until hearing returned (Musiek, 2006). These treatments have been replaced by newer methods, including cochlear implants. Doctors place these implants into a patient's ears through a surgical procedure involving an external microphone that is connected to an internal electrode placed on the auditory nerve. This produces hearing sensations similar to those produced from normal auditory cells (Moser, 2006). Digital hearing aids are another treatment available for patients suffering from milder cases of hearing impairment. These hearing aids receive sound through a microphone and convert it to electrical signals in the device. The signals are intensified by an amplifier, which sends the sound to the ear via the speaker. Furthermore, personal frequency modulation systems can be used to improve hearing quality. These are comprised of a speaker used by an orator and a receiver used by the listener (Carmen, 2004). Sound is amplified for the listener and improves the quality of hearing.

The work of Georg von Békésy played a critical role in the development of the biological model of the ear and also in understanding the workings of the inner ear. By 1928, he had developed anatomical techniques that allowed for quick, non-damaging dissection of the cochlea. In the 1930's, he demonstrated that the basilar membrane of the cochlea has frequency selective properties (Bekesy, 1989). While many of his experimental conclusions have since been challenged, his pioneering efforts led to further knowledge and development in the auditory field.

In the late 1970s, David Kemp expanded on Békésy's work and discovered otoacoustic emissions, which are low intensity sounds produced by the cochlea in response to stimuli (Kemp, 2000). These findings led him to develop hearing tests for infants by monitoring the levels of these emissions. More recently, studies have focused on identification of the genes responsible for inner ear function and development (Heller, 2002). This research concluded that mutations interrupt the auditory pathway. Detailed examination of mutations occurring on genes linked with deafness will provide a more complete picture of how hearing loss occurs.

Among the many genes that contribute to deafness, four specific genes are targeted in this review. While it has long been known that the genes studied affect many of the biological workings within the cell, they have only recently been linked to deafness (Morton, 2002). Advancements in research for these four genes have led to a better understanding of the mechanisms functioning in the auditory system. In this study, we will examine these four genes due to their essentiality in normal hearing.

Exploration of four genes involved in nonsyndromic deafness reveals the impact of gene

*These authors wrote the paper for Biology 221: Cell and Molecular Biology, taught by Dr. Shubhik K. DebBurman.

mutations on the auditory system function. After moving through the outer and middle ear, sound waves enter the cochlea, causing the tectorial membrane to vibrate (Musiek, 2006). This movement is picked up in the hair cells' stereocilia, where the cell will later convert it to chemical signaling. One of the most critical genes involved with stereocilia is Myosin VI, which is essential for their maintainance and stabilization.

Myosin VI

An Experienced Traveler
Myosin VI, a motor protein, is found on the inner and outer hair cells of the cochlea. It is especially concentrated in the cuticular plates and pericuticular necklace, which is present in the hair cell cytoplasm (Figure 1). Myosin VI is also located at the base of microvilli, which is made up of bundles of actin filaments. The location of Myosin VI is critical to the function of protein in stability of hair cell stereocilia (Self, et al., 1999).

Shifting Gears: Putting it in Reverse
Myosin VI is a very unconventional motor protein because it has a domain that binds an actin filament with two different ends. The barbed end of the filament is attached to the plasma membrane of a cell and is positively charged. The pointed end is negatively charged and reaches towards the inside of the cell (Ahmed, 2003). Typically, proteins of the myosin family move towards the positive end of the actin filament, but the unconventional Myosin VI moves towards the negative end (Hasson, 2003). This orientation enables the protein to transport away from the cell membrane, down the microvilli, and towards the cell.

Road Trip: The Mobility Mechanism
This method of mobility uses a specific mechanism. The protein produces movement on each particular filament by hydrolyzing ATP. In this process, ATP binds to Myosin VI, hydrolysis occurs, and previously bound ATP molecules are released as ADP and phosphate, providing the energy needed for movement. This hydrolysis reaction allows the protein to move the lever arm along the filament towards the negatively polarized end (Hasson, 2003).

Jamming on the Breaks: Restricted Movement
Recently, this movement was found to be essential for stabilization and organization of the hair cell stereocilia. At birth, wild type mice have normal organization of microvilli on the hair cells of the ear. Each hair cell has many differentiated microvilli protruding from the cell. Eventually, these microvilli develop into stereocilia bundles, which also appear as fingerlike projections from the hair cell and aid hair cell function (Hasson, 2003).

Traffic Jam: Stereocilia Fusion
Mutant mice are also born with mostly organized microvilli that undgo the transition to stereocilia. However, as development occurs, disorganization of the hair cells begins to increase. By three days after birth (DAB), most of the hair cells are affected by the process of fusion and excess growth of stereocilia. As stereocilia fuse together, the once differentiated stereocilia are reduced to only a few large protrusions from the hair cell (Figure 1B). By 20 DAB, there were no hair cells with normal stereocilia remaining in the cochlear duct. These cells fused together to create a large mass (Hasson, 2003). Research determined that mice lacking Myosin VI had fused stereocilia and did not respond to sound stimuli. This led to a further exploration of the role of Myosin VI in the auditory system.

Myosin VI is essential for maintenance of the stereocilia structures located on the apical surface of the hair cells. This function is specific to Myosin VI, as mutations in other myosin proteins do not yield the same results (Hasson, 2003). The area of focus was centered on the stabilization of the stereocilia, because these cells are where the initial defect occurs in organisms with mutated Myosin VI genes. More specifically, the interaction of Myosin VI with the heavy concentration of actin at the apical region is significant (Hasson, 2003).

Myosin VI uses its two ends to complete different functions. The tail domain attaches to the apical membranes found between the stereocilia, while the motor domain anchors this membrane-stereocilia structure to the actin in the cuticular plate. When Myosin VI is absent, the apical membrane is poorly anchored and rises up in response to surface tension among the cells. This causes the stereocilia to zip up until there is no differentiation remaining (Self, 1999).
At the molecular level, mutations hinder the progressive movement of Myosin VI and lead to human autosomal dominant deafness. For example, a missense mutation of Cys^{442} to Tyr in the Myosin VI gene alters the ATP hydrolysis mechanism and the motor functions of the protein. Previously, the ADP dissociation rate was thought to be the limiting factor in the hydrolysis reaction, which then limited the amount of movement possible for Myosin VI. More recently, however, it was determined that the protein moves great distances without dissociating; therefore, ADP dissociation is not the rate limiting step. Additionally, ADP dissociation is increased by the mutation of Cys^{442}, further supporting these latest findings. The rate limiting step was actually determined as the conversion of ATP to ADP and phosphate. The limiting factor of the Myosin VI protein that occurs in mutated organisms makes it useless for anchoring the membrane and transporting cargo (Sato, 2004). The ability of the Myosin VI gene to complete its function of stabilizing the stereocilia of the hair cells is hindered (Figure 2). Evidence shows that mutants had no stimulus-related cochlear response even at very high sound intensities, which is considered profound hearing impairment (Self, 1999).

Put It in Park: The Anchor Mechanism
In mutants, the microvilli must have another anchoring mechanism that is sufficient for early development, but cannot prevent membrane fusion after they have become stereocilia. It is critical for the microvilli to be present in organisms during early development, as they are essential for the function of many organs (Self, 1999). Eventual loss of stereocilia has less impact than complete lack of microvilli, thus enabling the mutants to survive, providing they initially possess microvilli.

Where Do We Go From Here?
Myosin VI is a motor protein that anchors and stabilizes the stereocilia. When functioning normally, Myosin VI prevents fusion of the stereocilia, allowing

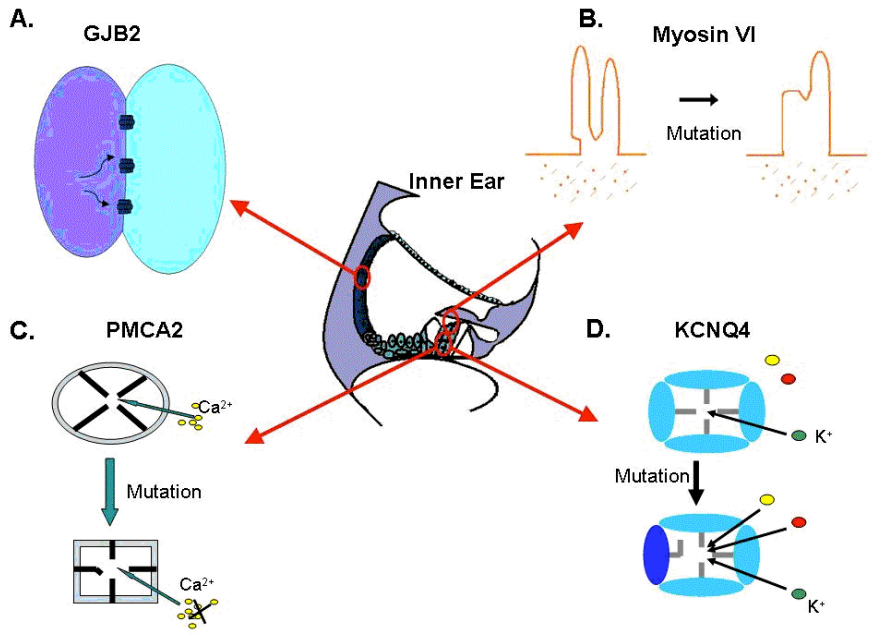

Figure 1. Gene Location in the Inner Ear. In the center of the figure is a diagram of the inner ear. Radiating from the various locations within the inner ear are the four genes covered in this review. In the four corners are illustrations of the four genes and their functions.

them to open the membrane channels and enacting an action potential. As the potential moves down the cell, Ca^{2+} must enter to release the chemical signal. The PMCA2 gene plays a critical role in the formation and function of the Ca^{2+} channels.

PMCA2

Go With the Flow: Ion Trafficking

The influx of Ca^{2+} ions is necessary for vesicles to bind to the presynaptic membrane and neurotransmitters to be released. The surge of calcium ions is controlled by the plasma membrane Ca^{2+} PMCA (ATPase), a transport protein in the plasma membrane of cells that brings calcium into the cell. There are four basic isoforms of PMCA pump: PMCA1, PMCA2, PMCA3 and PMCA4 (Grati et al, 2006). PMCA1 and PMCA4 are expressed in many different tissues and cell types, whereas PMCA2 (also known as ATP2b2) and PMCA3 operate in specialized tissues, such as brain and muscle tissues (Grati et al, 2006). PMCA2 demonstrates a highly restricted tissue distribution, suggesting that PMCA2 serves a more specialized and physiological function than the other isoforms.

Specifically, PMCA2 is thought to be present in high levels on the cell membrane of the outer hair cells (OHC). PMCA2 plays an essential role for hearing in relation to its function in both the vestibular and auditory systems. The main function of the PMCA2 protein is to bring Ca^{2+} ions into the cell. Thus, a mutation in this protein could cause a Ca^{2+} shortage in the cell, which would disable the vesicles from binding to the presynaptic membrane and would cause a significant loss of hearing (Figure 1C).

Mutation: Takes Its Toll

A point mutation in the PMCA2 gene, in which adenine is substituted with guanine, causes a glycine-to-serine substitution (Street et al, 1998). In mice that have this missense mutation, PMCA2 is no longer localized in the stereocilia and the basolateral membrane of hair cells, as normally seen in wild-type mice. This indicates that a glycine-to-serine substitution on the PMCA2 protein may cause deafness and imbalance (Street et al, 1998).

The particular role of PMCA2 in hearing was examined by observing PMCA2 deficient mice. At five weeks of age, these mice demonstrated imbalance of movement (Kozel et al, 1998). They also showed an underdevelopment of the organ of Corti canal, as well as the hair and the pillar cells (Kozel et al, 1998). This suggests that a PMCA2 deficiency is connected to structural changes in the organ of Corti tunnel (Figure 2).

Additionally, the PMCA2 deficient mice were examined by an Auditory Brainstem Response (ABR) test, which demonstrated that the PMCA2 deficient mice showed no response to varying intensities of sound (Kozel et al, 1998). However, the wild-type mice demonstrated a normal response to varying intensities of sound (Kozel et al, 1998). This indicates that a lack of PMCA2 causes severe imbalance as well as a significant loss of hearing.

Lastly, the effects of ablation on the pump were examined. There are two locations at which the ablations could occur, site A and site C. Site A is located in the cytosolic loop and site C is located in the C-Terminal, or the tail of the pump (Ficarella et al, 2007). When the cells were perfused with calcium chloride, mutations at Site A and Site C caused a dramatic decrease in the intake of Ca^{2+} ions (Ficarella et al, 2007). Thus, it can be deduced that PMCA2 plays a critical role in the intake of Ca^{2+} ions, which implies that a decrease in the ion concentration leads to a significant loss of hearing.

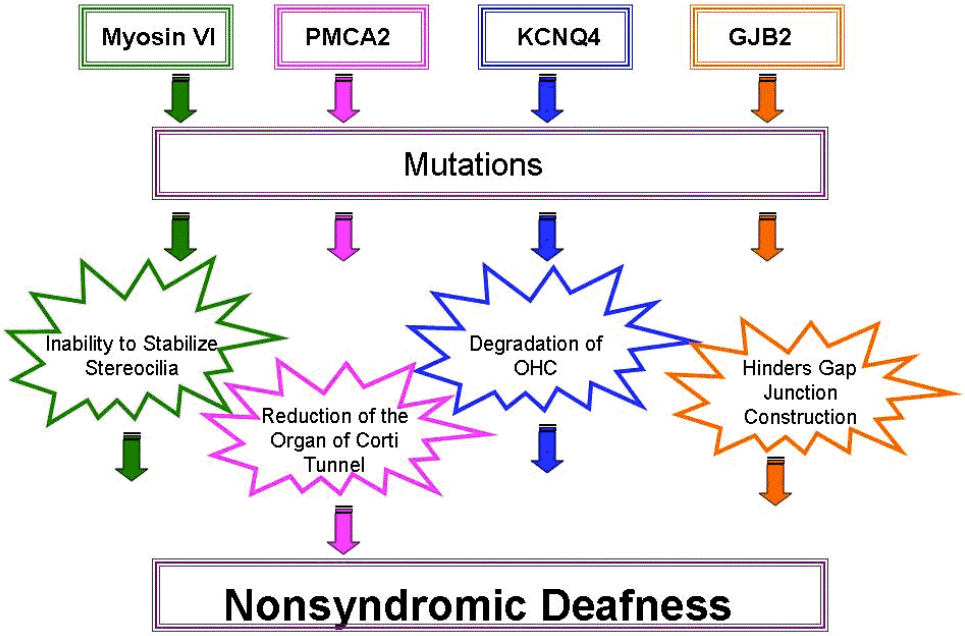

Figure 2. Effects of genetic mutations. This diagram illustrates the four specific genes studied and the effects of their mutation. Each gene mutation causes malfunctions of a particular area within the inner ear. Alterations of any one of these four genes can lead to a defect in specific proteins. These proteins then lead to the deformation of cell structure, resulting in nonsyndromic deafness.

Expressway to Deafness

Recent research and experimentation have shown that malfunction of the PMCA2 leads to a decrease in Ca^{2+} entering the cell, significant imbalance, and a loss of hearing. However, if PMCA2 gene is functioning normally, then a Ca^{2+} influx causes the vesicle to bind to the presynaptic membrane and to release the neurotransmitter.

At this point, the electrical signal has departed the cell. The sensory cells need to prepare for the next signal by returning to resting potential. The cell does this by forming ion channels that remove the K^+ buildup. KCNQ4 is essential for the formation of these types of ion channels.

KCNQ4

The Beginning of the Roadtrip: Background on Voltage-Gated Potassium Channels

In the mid 1990's, only two genes were known to encode K^+ channels, *KCNQ1* and *KCNE1* (Kubisch et al, 1999). KCNQ1 is a voltage-gated K^+ channel with six transmembrane domains, found in the cochlea and other locations, including the heart and brain cells. KCNQ1 helps in the secretion of K^+ into the endolymph from the stria vascularis. KCNE1 further enhances the currents from KCNQ1. Mutations in either protein halts the K^+ recirculation (Beisel et al., 2005) and can lead to a disease known as long QT syndrome. This disease is characterized by arrhythmias due to repolarization anomalies of the cardiac action potential, and can lead to sudden death (Kubisch et al, 1999). Two other genes, *KCNQ2* and *KCNQ3*, also create K^+ channels, but are not specific to the ear. Neonatal convulsions result from mutations in either of these proteins (Kubisch et al., 1999). Recently, the KCNQ protein family has been found in many more electrically excitable tissues, including skeletal muscle and the retina (Beisel et al., 2005).

An Unknown Route: The Identification of KCNQ4

In 1999, a new gene was discovered that belonged in the KCNQ family of K^+ channels. This gene, *KCNQ4*, was specifically found in the outer hair cells of the cochlea and was mapped on the DFNA2 locus (Kubisch et al, 1999). This area also encodes many other proteins, which, when mutated, cause diseases associated with dominant progressive hearing loss (Figure 2). Therefore, KCNQ4 was a likely candidate for autosomal dominant high-frequency hearing loss.

A Well-Oiled Vehicle: The Function of KCNQ4

In non-mutated genes, KCNQ4 forms tetramers in which four proteins join to form the pore. In each of these proteins, a region called the P-loop forms the narrowest part. This narrow section allows the pore to be selective for K^+ ions (Kubisch et al, 1999). After the influx of calcium, the voltage-gated KCNQ4 channels open to allow the passive diffusion of K^+ out into the perilymph, repolarizing the cell (Wangemann, 2006). This K^+ current out of the hair cells is then picked up by the surrounding cells and recycled back into the endolymph.

Road Construction Ahead: A Mutation in the Genetic Sequence

In a French family with high-frequency deafness, a mutation (G285S) was found in the sequence for the P-loop for the K^+ pore (Kubisch et al, 1999). The normal amino acid sequence contains glycine, which is highly conserved in all the KCNQ proteins (Kubisch et al., 1999). However, the mutant gene sequence had a change of one specific nucleotide, replacing guanine with adenine. From this, the glycine amino acid is converted to a serine (Kubisch et al, 1999). This

mutation disrupts the selectivity of the pore, and causes it to function improperly (figure 1D). When the wild-type (WT) and mutant proteins were expressed in *Xenopus oocytes*, the K^+ current out of the cell drastically decreased in mutant channels compared to the WT channels (Kubisch et al, 1999). From these results, it was believed that KCNQ4 was important in the removal of K^+ for keeping the electrical excitation conditions (the concentration of K^+ in the endolymph) constant for the next round of stimulation.

Triple A: Developing the Mouse Model

At that time, the only models available to study channels were frog eggs, which are very different from sensory transduction cells. Therefore, an advanced model was needed to better understand the function of the proteins on the OHC, and a mouse model was created. Researchers were able to produce knockout mice (KO) which deleted the P-loop region, and knock-in mice (KI) that had the specific mutation in the genome (dominant negative allele). It was found that the knockout and dominant negative allele mice had the same reduction for the K^+ current out of the cells at 3 weeks of age. Over a longer period of time, both types of mice had the OHC completely degraded (Kharkovets et al., 2006). By missing an essential part of the hearing pathway (the OHC), the signal was not able to be passed on, and thus deafness occurred. Currently, it is believed that KCNQ4 is essential in the OHC to remove the K^+ ions in order to repolarize the cell, not to replenish the endolymph K^+ concentration as originally thought.

A mutation in the KCNQ4 gene leads to an amino acid change in the K^+ pore and results in a nonselective ion channel (Figure 1D). The cell is unable to repolarize, and degration of the cell occurs over time. If the KCNQ4 gene is normal, the K^+ channel is able to return the cell to resting potential, and the next signal can be generated.

Another important part in generating the next signal is the recycling of K^+. While returning to resting potential, the hair cells excrete the K^+ ions into the perilymph. However, the ions must go back to the endolymph to be used in a future action potential. The use of intercellular connections made by connexins is critical for this movement.

Connexin 26

Conjunction Junction, What's Your Function: Location and Normal Function of Connexin 26

Connexin 26, a gap junction protein found in the cochlea and epidermis, is created with instructions found on the GJB2 gene. More specifically, connexin 26 is found in the stria vascularis, the spiral limbus, and the supporting cells found in the cochlea (Figure 1A).

Connexins are a family of transmembrane proteins that form channels allowing for rapid transport of ions and small molecules. Intercellular communication relies on gap junctions created by proteins like connexin 26. Channels formed with connexin 26 are largely involved in the transport of potassium and other small molecules (Casalotti et al., 1999). Potassium is required for the transformation of sound waves into electrical impulses that can be sent from cell to cell. The importance of potassium in hearing and influence of connexin 26 on potassium levels has lead researchers to explore the link between connexin 26 and deafness.

At least four other connexins are also found in the ear, some of which are also associated with deafness. Connexin 26 mutations, however, are the most prominent cause of nonsyndromic forms of deafness. Deafness related to connexin 26 occurs in an autosomal recessive manner (Ahmad et al., 2005). Different mutations in the GJB2 gene lead to various problems involving function, but the outcome is the same in all cases: profound deafness. While researchers know that GJB2 mutations lead to deafness, the way in which GJB2 mutations affect hearing remains largely unknown. Several factors have made studying GJB2 mutations difficult. The biggest hindrance to research is the fact that GJB2 deficient mice are embryonically lethal (Aoki et al., 2003). Thus, transgenic mice are the key to understanding deafness as caused by GJB2 mutations.

Potholes in the Road: The Effects of Mutations

Mutation of the GJB2 gene was found to generate significant changes in the inner ear of mutant mice. Hearing development in mice differs from that of humans, in that humans have fully developed hearing capabilities at birth, while mice are not fully matured in this area until about two weeks after birth (Aoki et al., 2003). Examination of the inner ear of the genetically mutated mice at two weeks revealed that the tunnel of Corti had collapsed and that sensory hair cells were deformed. By seven weeks the organ of Corti had begun to degenerate as had the spiral ganglia and hair cells. However, components responsible for the generation of endocochlear potential, such as the spiral ligament and stria vascularis, showed no alteration (Aoki et al., 2003).

The sustained structural integrity of these components suggests that the conditions within the endolymph have been maintained. This led researchers to believe that the epithelial gap junction system as the malfunctioning system. Disruption of the epithelial gap junction system hinders potassium intake which is necessary for transmission of signals by the hair cells (Aoki et al., 2003).

In addition to destroying or damaging many of the necessary inner ear components, various GJB2 mutations have been seen to impair intercellular coupling. Gap junctions are thought to be involved in the circulation of potassium between the fluids of the inner ear (Ahmad et al., 2005). Thus, understanding how and where mutations interfere with the formation of gap junctions is an important step in moving towards treatment. Connexin 26 is comprised of two transmembrane domains and extracellular loops. Alterations in these respective areas produced different results (Casalotti et al, 1999). Mutations in the transmembrane domains prevented oligomerization of proteins, thereby hindering the formation of connexons. Other mutations disrupt the alignment of the connexons or the structure of the gap. Problems with connexons or their formations interfere with intercellular coupling (Figure 2). Certain mutations affect the flow of biochemicals while leaving the flow of ions unharmed (Ahmad, 2005).

Around the Bend: Future Studies in Deafness

Deafness is a pathological condition that causes partial or complete loss of hearing. Many genes responsible for this disease were identified and four specific genes were investigated. Although current studies on deafness have helped to fill some of the gaps in knowledge, the precise mechanisms involved in hearing are still not completely understood. The inner ear is a

complex organ involving many cells with varying functions. While individual cells and their components seem like small tasks to investigate, their effects on the system as a whole are much more difficult to understand.

This review examined four genes, but over thirty different genes have been linked to deafness. The complexity of the four genes reviewed helps illustrate how complicated the picture truly is. Mutations on any of these thirty genes lead to profound changes that hinder other processes as well, leading to the degradation of sound perception.

Despite the fact that recent research has discovered much about these four genes, many questions still remain. The rate step of the ATP hydrolysis mechanism and its effects on hearing have many gaps that need to be explored to truly understand the role of Myosin VI in the auditory system. Additional studies could examine the overall understanding of the mechanism underlying the histopathology of the organ of Corti and how PMCA2 is dependent on other ion channels. It is still not clear how the build up of K^+ in the hair cells leads to the degradation.

Further understanding of how and why the cell responds to the high K^+ concentration is key to understanding another step in deafness. Also, the mouse model created in recent years can provide a useful tool for testing drugs (like K^+ channel openers) to slow the progress of deafness (Kharkovets et al., 2006). One recent study illustrated how Connexin30-linked deafness could be combated by increasing the expression of Connexin 26 (Ahmad, 2007). Future studies could reverse this experiment and explore correcting Connexin 26-linked deafness through the use of other connexins, such as connexin30.

The Final Destination

Many genes play a critical role in auditory system function. The four genes described each play a different role in hearing, but share a common theme. Mutation of each gene causes a significant disruption of hearing (Figure 2). Myosin VI gene mutations affect the mobility of proteins, which in turn affects the stability of the stereocilia. Mutations in the PMCA2 pump cause a significant loss of hearing, imbalance, and a dramatic decrease in calcium's entrance into the cell. KCNQ4 mutations reduce the ability to repolarize the hair cells resulting in the cells degradation. Connexin 26 is prevalent throughout the inner ear and plays a vital role in the formation of gap junctions, which allow for the transport of potassium ions. While each gene performs a very different function, they all play a critical role in the auditory system.

Acknowledgments

We would like to thank Dr.DebBurman for his guidance throughout the project. We would also like to thank Lisa Jeziorny, Lindsey McCurdy, Katie Michael, Jenny Riddle, and Kim Silvers for providing us with their example of this project. We would like to express our appreciation to Lisa Jeziorny and Lokesh Kukreja for their assistance in proofreading our work.

References

Ahmed, Z., Morell, R., Riazuddin, S., Gropman, A., Shaukat, S., Ahmad, M., Mohiddin, S., Fananapazir, L., Caruso, R., Husnain, T., Khan, S., Riazuddin, S., Griffith, A., Friedman, T., Wilcox, E. (2003). Mutations of MY06 are associated with recessive deafness, DFNB37. *Am. J. Hum. Genet., 72*, 1315-1322.

Ahmad, S., Chen, P., Lin, X., Sipp, J.A., Tang, W., Zhang, Y. (2005). Gap junction-mediation intercellular biochemical coupling in cochlear supporting cells is required for normal cochlear functions. PNAS. 102(42), 15201-15206.

Ahmad, S., Chang, Q., Chen, P., Hibshman, J., Li, Y., Lin, X., Qu, Y., Sohl, G., Tang, W., Willecke, K. (2007). Restoration of connexin26 protein level in the cochlea completely rescues hearing in a mouse model of human connexin30-linked deafness. PNAS. 104, 1337-1341.

Aoki, Y., Ichinohe, A., Ikeda, K., Katori, Y., Kobayashi, T., Kojima, K., Kudo, T., Kure, S., Matsubara, Y., Suzuki, M., Suzuki, Y., Xia, A. (2003). Transgenic expression of a dominant-negative connexin26 causes degeneration of the organ of Corti and non-syndromic deafness. Human Molecular Genetics 12(9), 995-1004.

Beisel, K., Rocha-Sanchez, S., Morris, K., Nie, L., Feng, F., Kachar, B., Yamoah, E., and Fritzsch, B. (2005). Differential expression of KCNQ4 in inner hair cells and sensory neurons in the basis of progressive high-frequency hearing loss. *J. Neurosci, 25*, 9285-9293.

Bekesy, G. (1989). Experiments in Hearing. *Acoustical Society of America.*

Carmen, R. (2004). The Consumer Handbook on Hearing Loss and Hearing Aids: A Bridge to Healing. *Auricle Ink Publishers.*

Cassalotti, S., Coleman, S., Evans, H., Forge, A., Martin, P. Properties of connexin26 gap junctional proteins derived from mutations associated with non-syndromal hereditary deafness. (1999). Human Molecular Genetics 8(13), 2369-2376.

Ficarekka. R., Di Leva. F., Bortollozzi. M., Ortolano. S., Donaudy. F., Petrillo. M., Melchionda. S., Lelli. A., Domi. T., Fedrizzi. L., Lim. D., Shull. G., Garpaini. P., Brini. M., Mammano. F., and Carafoli. E. (2007). A functional study of plasma-membrane calcium-pump isoform 2 mutants causing digenic deafness. *PNAS, Vol. 104,* 1516-1521.

Hasson, T. (2003). Myosin VI: two distinct roles in endocytosis. *Journal of Cell Sciences. 116*: 3453-3461.

Hawkins, D., Lovett, M. (2004). The developmental genetics of auditory hair cells. *Human Molecular Genetics, 13*, R289-R296.

Heller, S., (2002). Application of physiological genomics to the study of hearing disorders. *J Physiology, 543*, 1-12.

Kemp, D. (2000). The Quantum of Damages. *Thompson Professional Pub Cn, 4.*

Kharkovets, T., Dedek, k., Maier, H., Schweizer, M ., Khimich, D., Nouvian, R., Vardanvan, V., Leuwer, R., Moser, T., and Jentsch, T. (2006). Mice with altered KCNQ4 K+ channels implicate sensory outer hair cells in human progressive deafness. *EMBO J. 25*, 642-652.

Kozel. P., Friedman. R., Lawrence. E., Ebenezer. Y., Liu. Lynne., Riddle. T., and Shull. G. (1998). Balance and hearing deficits in mice with a null mutation in gene encoding plasma membrane Ca^{2+} ATPase isoform 2. *Journal of Bilogical-Chemistry, Vol. 273,* 18693-18696.

Kubisch, C., Schroeder, B., Friedrich, T., Lutjohann, B., El-Amraoui, A., Marlin, S., Petit, C., Jentsch, T. (1999). KCNQ4, a novel potassium channel expressed in sensory outer hair cell, is mutated in dominant deafness. *Cell 96*, 437-446.

M'hamed. G., Aggarwal. N., Emanuel. S., Wenthold. R. (2006). Molecular determinants for differential membrane trafficking of PMCA1 and PMCA2 in mammalian hair cells. *Cell, Vol. 119*, 2995-3007.

Morton, C. (2002). Genetics, genomics and gene discover in the auditory system. *Human Molecular Genetics, 11*. 1229-140.

Moser. T., Bramdt. A., Lysakowski, A. (2006). Hair cell ribbon synapses. *Cell Tissue Res, 326*, 347-359.

Musiek, F., Baran, J. (2006). The Auditory System: Anatomy, Physiology, and Clinical Correlates. *Allyn and Bacon.*

Piatto, V., Nascimento, E., Alexandrino, F., Oliveira, C., Lopes, A., Sartorato, E., Maniglia, J. (2005). Molecular genetics of non-syndromic deafness. *Rev Bras Otorrinolaringol, 71*, 216-23.

Redowicz, M. (2002). Myosins and pathology: genetics and biology. *Acta Biochimica Polonica, 49*, 789-804.

Schrijver, I. (2004). Hereditary non-syndromic sensorineural hearing loss. *J Molecular Diagnostics, 6*, 275-284.

Self, T., Role of Myosin VI in the differentiation of cochlear hair cells. (1999). *Developmental Biology. 214*: 331-34.

Sato, O., White, H., Inoue, A., Belknap, B., Ikebe, R., Ikebe, M., (2004), Human deafness mutation of Myosin VI (C442Y) accelerates the ADP dissociation rate. *Journal of Biological Chemistry*. 279: 28844-28854.

Street. V., McKee-Johnson. J., Fonseca. Rosalia., Temper. Bruce., and Noben-Trauth. K. (1998). Mutations in a plasma membrane Ca^{2+} ATPase gene cause deafness in deafwaddler mice. *Nature, Vol. 19*, 390-394.

Wangemann, P. (2006). Supporting sensory transduction: cochlear fluid homeostasis and the endocochlear potential. *J Physiol 576*, 11-21.