

TITLE: Genetic Hearing Loss

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INTRODUCTION

Hearing loss is the most common sensory deficit in humans. Roughly one child in a thousand is born with hearing impairment significant enough to compromise the development of normal language skills. Hearing loss can be caused by environmental factors as well as genetic factors. Environmental causes include pre- and post natal infection and ototoxic drug exposure. But it is estimated that 50% to 75% of all childhood deafness is due to hereditary causes. There are two main forms of genetic hearing loss, syndromic and nonsyndromic. Children with syndromic hearing loss have other clinical features in addition to the hearing loss. About 15-30% of the hereditary hearing loss is syndromic, while the vast majority is nonsyndromic (70%).

Hereditary hearing loss can be transmitted in several inheritance patterns, including autosomal dominant, autosomal recessive, X-linked inheritance and mitochondrial inheritance. Briefly, autosomal dominant inheritance exhibits a vertical pattern of transmission. Only one altered gene is needed for an individual to be affected. The offspring has 50% chance to receive the copy of the mutated gene from the affected parent. The most common pattern of transmission in hereditary hearing loss is autosomal recessive. A child must have both copies of the mutated gene to exhibit deafness. The parents will most likely have normal or near normal hearing even though they possess the recessive gene. Typically there is a 25% chance that the offspring will be affected and manifest hearing impairment or deafness. This mode of transmission has a horizontal pattern. The X-linked inheritance involves particular genes located on the X chromosome. It more commonly affects male because they possess a single X chromosome and will present phenotypically with any mutation on the X chromosome. Female can carry the mutation on one of the X chromosomes without phenotypic expression. Her sons have a 50% chance of inheriting the mutation and express phenotypically. The daughters have a 50% chance of inheriting the mutation and become a carrier of the mutation. Mitochondrial inheritance is caused by mutations in the mitochondrion DNA, small loop of DNA in mitochondrion. Only mothers can pass on the mutation because only the eggs carry mitochondrion DNA.

When two genetic loci lie near each other on a chromosome, they tend to be inherited

together. Based on this principle, genetic linkage analysis is used to localize the disease gene to a specific region on a specific chromosome. There are also hundreds of mouse models available for studying genetic hearing loss. Each provides a piece of the puzzle in our understanding of inner ear biology. Some are directly relevant to human deafness and others provide key elements in the development and function of sensory structures of the ear.

Hereditary deafness is genetically a highly heterogeneous disease with many different genes responsible for auditory dysfunction. Genetic heterogeneity has been observed repeatedly in our improved understanding of syndromic hearing loss. Mutations of several different genes can cause the identical clinical phenotype. On the other hand, different mutations in one gene can cause variable phenotypes.

Syndromic hearing loss

There are over four hundreds syndromes with hearing loss have been described. The Online Mendelian Inheritance in Man (www.ncbi.nlm.nih.gov/Omim) has comprehensive descriptions of the clinical features and molecular genetics of these syndromes as well as an all-inclusive list of references.

Alport syndrome

It is a hereditary disorder of basement membranes. Majority of the cases are inherited in an X-linked manner (80%). Autosomal recessive as well as autosomal dominant inheritance has also been observed. It is characterized by the association of renal dysfunction with hearing loss. Symptoms include high frequency sensorineural hearing loss, hematuria with progressive renal failure, and ocular abnormalities. Microscopic hematuria was found to be the most reliable urinary criterion of hereditary nephritis in both males and females. The hematuria was often accompanied by red cell casts, indicating that the renal lesion is a glomerulitis. Men were more severely affected than women. They had striking urinary abnormalities in early childhood which progressed to renal failure in adulthood. Affected women had less obvious urinary findings and rarely developed uremia. Typical ocular associations are a dot-and-fleck retinopathy, which occurs in approximately 85% of affected adult males, anterior lenticonus, which occurs in approximately 25%, and the rare posterior polymorphous corneal dystrophy.

Mutations in three different type 4 collagen genes (*COL4A3*, *COL4A4* and *COL4A5*) have been identified. These collagens are found in the basilar membrane, parts of the spiral ligament, and stria vascularis. Although the mechanism of hearing loss is not known, it is suggested that the loss of integrity of the basement membrane might affect adhesion of the tectorial membrane. Also in the glomerulus there is focal thinning and thickening with eventual basement membrane splitting.

Branchial-oto-renal syndrome

It is an autosomal dominant disorder. The main clinical features include branchial derived anomalies, otologic anomalies, and renal malformation. The hearing loss found in BOR is highly variable. The loss can be sensorineural, conductive or mixed. It can be stable or progressive and the severity can range from mild to profound. Ear pits are found in about 80%

of the BOR cases. Of those persons with ear pits, some have only one while others have one in front of each ear. Some individuals have deformed outer-ear(s), middle-ear(s), and/or inner-ear(s). About 60% of the BOR cases have branchial cysts or fistulas which are small holes located on the front, external lower third of the neck. People with branchial cysts or fistulas usually have two (one on each side); however, some only have one. Because they often become infected, branchial cysts or fistulas are often surgically removed. Kidney anomalies are found in about 15% of those with BOR. Most of the anomalies have minimal clinical significance and consists of minor changes in the anatomy of the kidney or urine collection system. Kidney function is normal in these individuals. More severe kidney anomalies have been reported. This range from small, normal functioning kidneys all the way to the kidneys (one or both) being absent. Three of the less common symptoms associated with BOR are ear tags, blocked tear ducts, and short palate.

Branchio-oto-renal syndrome is caused by mutations in *EYAI* gene. Expression pattern of this gene indicated its role in development of the inner ear and kidney. *EYAI* homozygous knockout mice lack ears and kidneys because of apoptotic regression of the organ primordial.

Jervell and Lange-Nielsen syndrome

It is an autosomal recessive disorder. Persons affected by this syndrome have prolongation of the QT interval, torsade de pointe arrhythmias (turning of the points, in reference to the apparent alternating positive and negative QRS complexes), sudden syncopal episodes, and severe-to-profound sensorineural hearing loss.

Two genes, *KVLQTI* and *KCNE1*, have been identified. Little is known about the function of these genes. *KVLQTI* is expressed in the stria vascularis of mouse inner ear. Within the inner ear, endolymph homeostasis is controlled in part by the delayed rectifier potassium channel. This channel is formed by proteins encoded by the *KVLQTI* and *KCNE1* genes. Heterozygotes of *KVLQTI* have Romano-Ward syndrome, which unlike JLN syndrome, does not include severe-to-profound hearing loss in the phenotype.

Norrie syndrome

It is X-linked inheritance. Classic features include specific ocular symptoms (pseudotumor of the retina, retinal hyperplasia, hypoplasia and necrosis of the inner layer of the retina, cataracts, and phthisis bulbi), progressive sensorineural hearing loss, and mental disturbance, although less than one-half of patients are hearing impaired or mentally retarded.

NDP gene has been identified in association with this disease. It encodes for norrin, a protein that has homologies at the C-terminus to a group of proteins including mucins. The exact mechanism of hearing loss remains to be determined. It has been suggested that norrin protein regulates vascularization of the cochlea and retina.

Pendred syndrome

Pendred syndrome is the most common syndromal form of deafness, accounting for about 4-10% of cases. It is an autosomal recessive disorder and represents with goiter and

sensorineural hearing loss. Usually the goiter is clinically evident at approximately 8 years of age, but adult onset has also been noted. Patients are usually euthyroid but can be hypothyroid. A positive potassium perchlorate discharge test may be helpful in identifying an organification defect, the defective organic binding of iodine in the thyroid gland. But the test is nonspecific and its sensitivity is unknown. Hearing loss is usually congenital, bilateral, severe to profound, and sloping in the higher frequencies. It may be fluctuating or progressive. Variable vestibular dysfunction has been exhibited by the patients. Enlargement of the vestibular aqueducts and the endolymphatic sac and duct are found in nearly all patients. An incomplete partition of the cochlear may be present and the vestibule may be enlarged.

Mutations of the gene *PDS* on chromosome 7q31 account for most cases of Pendred syndrome. Mutations in this gene can also be found in nonsyndromic deafness DFNB4 and many cases of enlarged vestibular aqueduct syndrome. The *PDS* gene encodes pendrin, an anion transporter found in the inner ear, thyroid, and kidney. It possibly plays a role in endolymphatic fluid resorption.

A *PDS* knockout mouse was generated in 2001 by Everett, which showed complete deafness. The inner ear of the mouse model was anatomically normal until embryonic day 15, at which time all the endolymph-containing spaces start to enlarge. Inner and outer hair cell degeneration also observed under electron microscopic studies. Interestingly, no thyroid abnormalities have been observed with this mouse model.

Stickler syndrome

It is an autosomal dominant disease. Three phenotypes have been identified. Classic features of this syndrome include progressive myopia beginning in the first decade of life and resulting in retinal detachment and blindness, vitreoretinal degeneration, premature joint degeneration with abnormal epiphyseal development, midface hypoplasia, and irregularities of the vertebral bodies, cleft palate deformity and variable sensorineural hearing loss.

It is caused by mutations in *COL2A1*, *COL11A2*, or *COL11A1*. The classic phenotype is associated with mutations in *COL2A1*, a fibrillar collagen that is arrayed in quarter-staggered fashion to form fibers similar to those of COL1. Mutations in *COL11A2* cause STL3, a disease characterized by the typical facial features of STL1 in combination with hearing impairment. Cleft palate and mild arthropathy also occur, however ocular signs are absent.

Treacher Collins syndrome

Treacher Collins syndrome is a disorder of craniofacial development. It is autosomal dominant inheritance with variable expression. The features include slanting of the eyes with inferior displacement of the lateral canthi with respect to the medial canthi, coloboma of the lower lids, micrognathia, microtia and other deformity of the ears, hypoplastic zygomatic arches, and macrostomia. Conductive hearing loss and cleft palate are often present.

TCOF1 gene was identified. It has suggested that *TCOF1* plays a role in nucleolar-cytoplasmic transport. All of the mutations observed resulted in introduction of premature termination codons into the reading frame and cause premature termination of the protein product.

Usher syndrome

It is named for Charles Usher (1914), a British ophthalmologist who emphasized their hereditary nature. It is an autosomal recessive disorder. It is characterized by hearing loss and retinitis pigmentosa. Clinically three different types can be distinguished. They are distinguished based on the severity or progression of the hearing loss and extent of the vestibular system involvement. Type 1 patients have congenital profound congenital deafness, absent vestibular response and the onset of retinitis pigmentosa is in the first decade of life. Type 2 patients have normal vestibular response. Type 3 patients have progressive hearing loss, variable vestibular response and variable onset of retinitis pigmentosa.

A total of 11 loci and 6 genes have been identified in Usher syndrome. Among these, *MYO7A*, which belongs to the family of unconventional myosins, encode myosin 7A. It represents a unique molecular motor for hair cells. In the mutant mice, the organization of stereocilia bundles is disrupted, impairing the function of hair cells. *CDH23* gene encodes cadherin 23, which is an adhesion molecule maybe important for crosslinking of stereocilia. It may also be involved in maintaining the ionic composition of the endolymph. Studies have suggested that myosin 7A, cadherin 23, along with another protein harmonin, which is coded by *USH1c* gene, form a transient functional complex in stereocilia.

Waardenburg Syndrome

The disease was named for Petrus Johannes Waardenburg, a Dutch ophthalmologist (1886-1979) who was the first to notice that people with two different colored eyes frequently had hearing problems. The clinical features usually include dystopia canthorum, meaning the lateral displacement of the inner canthus of the eyes to give an appearance of a widened nasal bridge, pigmentary abnormalities of the skin, iris, and hair, and sensorineural hearing loss. There is a great deal of variation in the hearing loss. Some of the affected persons escape deafness. There are four subtypes of Waardenburg syndrome have been described. Type 1 typically shows wide confluent eyebrow, high broad nasal root, heterochromia irides, brilliant blue eyes, premature gray of hair, eyelashes, or eyebrows, white forelock, and vestibular dysfunction. Type 2 is like type 1 but without dystopia canthorum. The penetrance of hearing loss is slightly higher in type 2 than type 1. Type 3 is also called Klein-Waardenburg syndrome. It has type 1 clinical features and hypoplastic muscles and contractures of the upper limbs. Type 4 is also called Shal-Waardenburg syndrome. It has type 2 clinical features and Hirschsprung's disease.

Five genes on five chromosomes have been identified. *PAX3* gene mutations have been indicated to be associated with type 1 and type 3 phenotypes. *PAX3* is a DNA-binding protein that is important in determining the fate of neural crest cells in the developing nervous system. Type 2 has been linked to *MITF* gene mutation. Three genes have been associated with type 4 phenotypes. They are *EDN3*, *EDNRB*, and *SOX10*. In vitro studies of *EDN3* showed a stimulation of proliferation and melanogenesis of neural crest cells. *EDNRB* is postulated to play an essential role in the development of the two neural crest-derived cell lineages, epidermal melanocytes and enteric neurons. *SOX10* belongs to the family of transcription factors that bind DNA and regulate its transcription. Interaction of these three genes has been suggested. The genetics of Waardenburg syndrome highlight the principle of genetic heterogeneity.

Nonsyndromic hearing loss

According to the Hereditary Hearing Loss homepage (www.uia.ac.be/dnalab/hhh/), 80 loci for nonsyndromic hearing loss have been mapped to the human genome. And 30 genes have been identified. Based on the type of gene product, these genes can be categorized into several groups. 1) channel and gap junction components, 2) myosin and other cytoskeletal proteins, 3) transcription factors, 4) extracellular matrix proteins, 5) unknown function genes.

There are autosomal dominant, autosomal recessive and X-linked forms of nonsyndromic hearing loss. In general, recessive inheritance shows prelingual onset of hearing loss. And the severity is severe to profound with all frequencies affected. In autosomal dominant forms, the phenotype is less severe. The onset is usually postlingual. The severity is ranging from moderate to severe. Hearing loss is seen in middle, high, or all frequencies with only three loci having hearing loss in the low frequency range.

Genes for homeostasis

Maintaining ion homeostasis within the cochlear duct, especially the high potassium concentration in the endolymph, is of extreme importance for signal transduction involved in the hearing process. Potassium recycling pathway is postulated to start with an efflux of potassium from the outer hair cells through potassium channel. Through gap junctions between the supporting cells, the ions migrate to the stria vascularis, for where they are secreted into the endolymph.

Four connexins have been implicated: connexin 26 (*GJB2*), connexin 31 (*GJB3*), connexin 30 (*GJB6*), and connexin 43 (*GJA1*). Connexins belong to a family of gap junction proteins responsible for the intercellular transport of ions, metabolites, and second messengers. *GJB2* is the first nonsyndromic sensorineural deafness gene to be identified. It is estimated that mutations of *GJB2* accounts for 50% of recessive nonsyndromic hearing loss. *GJB2* encodes connexin 26. Animal studies to examine the role of connexins in the cochlea have been undertaken only for this protein. It is expressed in stria vascularis, basement membrane, limbus, and spiral prominence of cochlea. Rat immunohistochemical finding suggest that it plays a role in recycling of potassium ions back to the endolymph of the cochlear duct after stimulation of the sensory hair cells. 80 recessive and 6 dominant mutations have been found in *GJB2*. One mutation, 35delG (one guanosine residue deletion from nucleotide position 35), is very frequent in Caucasian population. This mutation results in shifting of the reading frame and protein truncation. Because of the high prevalence of this mutation in Western countries and the small size of the *Gjb2* gene, diagnostic testing is available.

KCNQ4 is a component of a potassium channel. It is thought to be involved in the potassium recycling pathway as well. *SLC26A4* encodes an anion transporter. This mutation gives rise to a broad spectrum of clinical symptoms: from nonsyndromic SNHL to hearing loss with enlarged vestibular aqueduct to full-blown Pendred syndrome with thyroid goiter.

Transcription factors

Four transcription factor genes have been identified: *POU3F4*, *POU4F3*, *EYA4*, and

TFCP2L3. *POU3F4* is responsible for X-linked mixed hearing loss. The conductive hearing loss is from stapes fixation. These patients suffer from an increased perilymphatic pressure causing the typical “gusher” that appears during stapes footplate surgery. *POU4F3* is associated with autosomal dominant hearing loss. Its knockout mice fail to develop hair cells with subsequent loss of spiral and vestibular ganglia. No gross morphological changes were seen in *Pou3f4* knockout mice, but ultrastructural alterations were found in the spiral ligament fibrocytes. Both *EYA 4* and *TFCP2L3* cause autosomal dominant hearing loss.

Cytoskeleton proteins

Maintaining the highly organized and specialized structures like actin-rich stereocilia of the sensory hair cells is very important for the processes of hearing and balance. Among the cytoskeletal components involved in nonsyndromic hearing loss are myosins, otoferlin, actin-polymerization protein, harmonin, and cadherin. Four unconventional myosins genes (*MYO3A*, *MYO6*, *MYO7A*, *MYO15*) and one conventional myosin gene *MYH9* have been identified. Myosins are molecular motor proteins that bind to actin and that hydrolyze ATP to generate the force to move across actin filaments. Unconventional myosins move macromolecules along actin filaments. They are important for the structural integrity of the stereocilia. All the unconventional myosin mouse models display the vestibular dysfunction.

Otoferlin acts in calcium triggered synaptic vesicle trafficking. It is encoded by *OTOF*. Recently it has been reported to account for 4.4% of recessive nonsyndromic hearing loss negative for *GJB 2* mutations in Spanish population. If this finding is confirmed in other population, it might be one of the genes with putative diagnostic implications.

Harmonin organizes multiprotein complexes in specific subcellular domains. They anchor and cluster transmembrane proteins and recruit signaling molecules. They are also associated with Usher type 1C disease.

Cadherins are components of adherens junctions and play critical roles during embryogenesis and organogenesis. It is encoded by *CDH23*. In mouse model, the mutations showed disruption of stereocilia organization during early hair cell differentiation. It is also associated with Usher type 1D disease.

Extracellular matrix components

The tectorial membrane overlying the hair cells plays a crucial role in the mechanosensory transduction process. *TECTA* gene encodes alpha tectorin, a component of the tectorial membrane. Knockout mice showed detachment of tectorial membrane from the cochlear epithelium. *COL11A2* encode type XI collagen. The knockout mice have atypical and disorganized collagen fibrils of the tectorial membrane. *COCH* protein is ubiquitously present in the inner ear. It is expressed in cochlea as well as vestibular organs. *COCH* mutation exhibits symptoms of Meniere, including vertigo, tinnitus and a pressure feeling in the ear.

Mitochondrial Hearing Loss

In addition to nuclear genes some mitochondrial genes have been associated with genetic hearing loss. As mitochondria have a crucial function in nearly every cell, it is not

unexpected that mitochondrial DNA mutations mainly cause multisystemic diseases, of which hearing impairment is often an additional symptom. Two different mitochondrial genes are involved. First, the 1555A->G mutation in the 12S rRNA gene leads to aminoglycoside-induced hearing loss and nonsyndromic hearing loss. The second gene is the tRNA^{Ser}(UCN) gene, in which four different mutations are known to cause nonsyndromic hearing impairment.

Evaluation

A complete history should include prenatal, perinatal, postnatal, and family history. Especially in family history, it is important to inquire hearing loss in the first and second degree relatives, especially if the loss started before age 30. Consanguinity or common origin from ethnically isolated areas should increase suspicion of hereditary hearing loss. If there are a number of family members with hearing loss, constructing a pedigree is important.

Physical exam should focus on looking for features associated with congenital infection or syndromic hearing loss. Note hair color, the presence of a white forelock, facial shape, and skull shape. On eye examination, one should note the color of the iris, position of the medial and lateral canthus, intercanthal distance, cataracts, and any retinal findings. Examine the ear for preauricular pit, skin tags, shape and size of the pinna, or abnormality of EAC or TM. Examine the neck for branchial anomalies and thyroid enlargement and oral cavity for cleft. Thorough inspection of skin for areas of hyper or hypopigmentation and café-au-lait spots. Do a complete neurological exam including gait and balance to evaluate vestibular function.

In addition to audiogram, lab test should be ordered based on the history and physical exam. All children with hearing loss should have a urinalysis to assess for renal dysfunction. Other tests should be ordered as appropriate, for example, thyroid function test and EKG in suspected Pendred syndrome and Jervell and Lange-Neilsen syndrome. A CT scan of temporal bone is the radiological test of choice for evaluation of pediatric sensorineural hearing loss and should be considered in all patients with progressive hearing loss and craniofacial anomalies. The most common finding is dilated vestibular aqueduct, followed by Mondini malformation. Dilated vestibular aqueduct (DVA) suggests the diagnosis of Pendred syndrome, but may be found in branchio-oto-renal syndrome or in isolation. All patients with documented DVA should be investigated for Pendred syndrome.

Genetic counseling is an important part of evaluation. The counseling team usually consists of clinical/medical geneticist, genetic counselor, social worker, and psychologists. The risk and benefit of genetic testing should be explained to the family and consent should be obtained before genetic testing. Three genetic tests are available: GJB2, SLC26A4, and EYA1. Knowing the genetic cause of a person's hearing loss can lead to improved decision about treatment and management. Genetic information can help predict whether the hearing loss will remain the same or whether it will worsen over time. Knowledge of the genetic cause is also helpful in determining what kind of damage has happened to the hearing system to cause the deafness. This is important because how the inner ear is damaged may affect whether a cochlear implant, or other hearing device, may help a patient. In addition, genetic testing can help determine if problems besides hearing loss may be present or may develop in the future. Genetic testing can also provide a deaf individual or the parents of deaf child information when making reproductive choices.

Conclusion

Since the identification of the first deafness gene in 1995 a respectable number of genes have been identified. As a consequence, the molecular knowledge of the processes responsible for hearing and balance and of the pathological mechanisms leading to hearing loss is expected to increase tremendously. But there is still a long way to go. Many genes remain to be identified. Moreover, the elucidation of the exact function of genes for which only a putative function was proposed, and of genes with an unknown function remains a great challenge.

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