GENETIC ASPECTS OF HEARING LOSS

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Genes mediate the workings of cells, organs and organisms. Since normal hearing is dependent on highly specialised structures and cellular functions it is not surprising that many genes – as well as environmental factors – affect this complex process. A number of genes have been identified to date that have added to our knowledge of the molecular aspects of hearing. Mutations, or changes, in these genes cause deafness or hearing impairment demonstrating that these genes are essential for normal hearing function. Despite the advances we have made in the discovery of "deafness" genes, little is known about the genes that determine susceptibility to noise-induced deafness, ototoxic hearing loss or early onset presbyacusis. Increasing our knowledge of the genetic aspects of hearing loss will lead to improved genetic counselling and will help the development of novel cell-based, gene or drug therapies.

1. INTRODUCTION

Hearing loss is the most common sensory condition, affecting approximately 10% of Australians. Although 1 in 800 newborn children have a hearing problem, it is espcially an issue for adults and the elderly: by the age of 65 to 75 years, half the population experience hearing impairment. The financial, social and personal costs of deafness to affected people, their families and the society are significant [1].

Deafness is an etiologically heterogenous trait caused by genetic and environmental factors. It is known that mutations in a number of genes can cause inherited hearing loss as can environmental factors such as infections, noise exposure, premature birth and exposure to ototoxic drugs. Hearing loss is usually classified as conductive or sensorineural. Conductive hearing loss is caused when sound waves are not able to pass through the outer or middle ear. It is often reversible, and can be caused by otitis media, otosclerosis, and presence of a foreign body or a tumour. Sensorineural hearing loss is usually irreversible, and commonly involves damage to or loss of cells in the inner ear (incl. hair cells) or auditory nerves. In most instances, hearing loss of genetic origin is sensorineural. Although we state that 60% of cases of childhood deafness are genetic and 40% environmental, some cases are caused by a combination of both genetic and environmental factors, eg. aminoglycoside-induced hearing loss. It is also true that genetics and environmental factors interact in the most common types of adult-onset hearing loss: presbyacusis and noise-induced hearing loss. Many genes are needed for correct auditory function. Genetic changes (mutations) that result in malfunction of an important "auditory" protein can result in deafness. It is estimated that there are several hundred such "auditory" genes [2].

2. GENETICS OF HEARING LOSS

2.1 Classification of inherited deafness

A syndrome is a combination of clinical features seen repeatedly in different individuals. When a person presents with hearing loss as their only clinical feature their deafness is classified as non-syndromic. If the deafness is only one of the clinical features in a syndrome it is classified as syndromic deafness. Hearing loss is a major feature of over 400 syndromes [3], Table 1.

Table 1. The 10 most common syndromes in which deafness is a major feature (from [30]).

Syndrome
Hemifacial microsomia
Stickler syndrome
Congenital cytomegalo virus
Usher syndrome
Branchio-oto-renal syndrome
Pendred syndrome
CHARGE Association
Neurofibromatosis type II
Mitochondrial disorders
Waardenburg syndrome

Inherited conditions are said to be either simple or complex. Simple genetic conditions follow Mendel's laws by showing typical and characteristic inheritance patterns (recessive, dominant, X chromosome linked, maternal). Complex genetic conditions are the result of interaction between many genes and environmental factors and therefore do not follow Mendelian inheritance patterns. Most of the common conditions that affect us are complex genetic traits. With many genes and environmental factors causing deafness one would assume that deafness belongs in the 'complex condition' category. That is normally not the case, and certainly not when investigating genetic deafness in newborns or young people. In such cases the inheritance pattern within a given family usually follows simple Mendelian laws. When looking at non-syndromic hearing loss the inheritance pattern is autosomal recessive in ~80%, autosomal dominant in ~15%, X-chromosome linked in ~3% and maternal (mitochondrial) in ~2% of families [4], but will vary somewhat depending on ethnic background.

2.2 Genes that cause deafness

The fact that inherited deafness in many families follows Mendel's laws makes identification of genes associated with hearing loss easier. Geneticists can – if a family has

enough affected members and is of a suitable structure – take advantage of recent developments in tracking genes (so-called linkage analysis or gene mapping) and in knowledge obtained in the human genome project. The human genome project has produced the DNA sequence and chromosome "address" of known and predicted human genes. To find genes associated with deafness, geneticists will often use linkage analysis to determine which chromosome and in which region on that chromosome a mutated gene is located. They will then search human genome data for candidate genes in the chromosome region and determine if any of these genes have a mutation that might explain the deafness. This can be a laborious task and in many cases a "deafness" gene is mapped to a chromosome region, but the gene itself has not been identified. So far > 80 genes associated with syndromic or non-syndromic hearing loss have been identified (Table 2). Information on these genes and links to more information can be found on The Hereditary Hearing Loss Homepage [5].

Rapid progress has been made in identifying "deafness" genes. How has the identification of "deafness" genes, helped our understanding of the molecular basis of auditory function? It has become clear that "deafness" genes code for proteins with a large range of functions, including structural proteins, transcription factors, enzymes in metabolic pathways, ion channels and ion transporters (Table 2). It is not the purpose of this article to describe these genes in detail, but we will mention two of the more important genes, connexin 26 and pendrin.

Connexin 26

With so many different genes being able to cause deafness, it was somewhat of a surprise to discover that one gene, the connexin 26 gene, is the cause of hearing loss in approximately 40% of

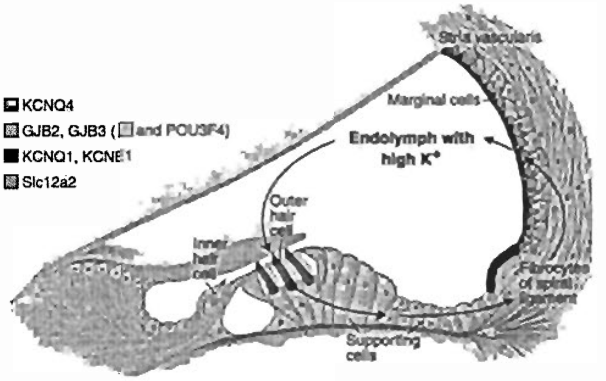

Figure 1 Potassium recycling in the cochlea. The route of potassium recycling from the outer hair cells through the supporting cells of the organ of Corti and fibrocytes of the spiral ligament. Na-K ATPase pumps, located in the marginal cells of the stria vascularis, pump the potassium ions back out into the endolymph. The anatomical localisation of the proteins encoded by common "deafness" genes in this pathway is depicted. From [8].

Australian children with prelingual, non-syndromic autosomal recessive hearing loss. We can calculate that approximately 1 in 50 Australians is an unaffected carrier of a connexin 26 mutation [6]. Connexin 26 mutations are some of the most common genetic mutations in our population [7]. Connexin 26 is a member of a family of transmembrane proteins that form intercellular gap junctions and whose function is to allow ions or small molecules to be transported between neighbouring cells. It is expressed in many tissues in the body, including the supporting cells, spiral ligament, fibrocytes and spiral limbus

Table 2. Examples of genes associated with hearing loss

Gene product	Type of deafness	Inheritance	Comments
Connexin 26	Non-syndromic	Recessive	The most common cause of prelingual inherited hearing loss. Connexin 26 caused deafness can in rare cases be syndromic or have dominant inheritance
Pendrin	Syndromic	Recessive	Pendrin mutations can also cause non-syndromic hearing loss
Mitochondrial 12S rRNA	Aminoglycoside induced	Maternal	
Myosin 7A	Non-syndromic or Syndromic	Dominant or Recessive	Myosin 7A mutations can cause Usher syndrome
Cadherin 23	Non-syndromic or Syndromic	Recessive	Cadherin 23 mutations can cause Usher syndrome
Harmonin	Non-syndromic or Syndromic	Recessive	Harmonin mutations can cause Usher syndrome
COCH	Non-syndromic	Dominant	Onset of deafness usually in teens
KCNQ4	Non-syndromic	Dominant	Voltage-gated potassium channel
PAX3	Syndromic	Dominant	PAX3 mutations can cause Waardenburg syndrome
SOX3	Syndromic	Dominant	SOX3 mutations can cause Waardenburg syndrome

of the cochlea [8]. It is thought that mutations in the connexin 26 gene disrupt the recycling of K+ back into the endolymph, thereby affecting endocochlear potential and/or cell viability.

Pendrin

Pendrin, or Solute carrier family 26 member 4 (SLC26A4), is another important gene associated with hearing loss. Studies suggest that pendrin mutations account for approximately 5% of all prelingual deafness [9]. Pendrin is an iodide-specific transporter. In the ear it is expressed throughout the endolymphatic duct and sac, as well as in nonsensory regions of the utricle, saccule and cochlea. These observations support the view that pendrin is involved in endolymphatic fluid resorption in the inner ear.

Mutations in the pendrin gene can cause a variety of clinical presentations. It was identified as the gene causing Pendred syndrome. However, mutations in the pendrin gene do not always cause goiter or cochlear malformations, such as Mondini dysplasia and enlarged vestibular aqueducts [10].

2.3 Why find genes for deafness?

The identification of "deafness" genes is a significant step forward in understanding the molecular basis of inner ear function. Although there are still large gaps in our knowledge, the study of genes coding for prestin, cadherins, harmonin, myosins, actins, TRP1 (the potential mechanoelectrical transduction channel) have – in combination with physiology and physics – given us unprecedented insight into cochlea function.

Identification of these genes also has clinical relevance [11]. Screening for connexin 26 mutations is now one of the most requested genetic tests. Identification of connexin 26 mutations in a family explains the cause of deafness, results in improved genetic counselling and has implications for prenatal and postnatal testing. In the future, new technology will make it practical and economically feasible to screen for mutations in many more "deafness" genes. One can also envisage that future treatments for hearing loss will target specific genes or functions.

2.4 Finding additional genes for hearing loss

Despite the recent successes, many more genes important in auditory function await identification and characterisation. Gene mapping and gene analysis of families affected by hearing loss have led to the identification of a number of "deafness" genes. For dominant conditions this requires large multigeneration families with many (usually >15) affected members. For recessive conditions large consanguineous families are often needed. Such families are not common in Australia, and the approach therefore not always an option. Most of the known genes associated with hearing loss are genes that cause early onset deafness. We know that there is a significant genetic contribution to the timing and severity of presbyacusis, but the genes and mechanisms involved are poorly understood, as are the genetic factors that influence the severity of a hearing loss.

How can we find these genes? Several approaches have been exploited by us and others in the hunt for the causative "deafness" genes. One such approach is to use microarray technology to gain insight into which genes are expressed at what level in specific structures of the inner ear or even in individual cells. Ideally one would compare gene expression between individuals with eg. early and late-onset presbyacusis, but because it is difficult to obtain human inner ear tissue, such comparisons are usually done on samples from animals, eg. mice [12; 13].

The analysis of mouse models for deafness is also a powerful approach to identifying human genes for hearing loss. The mouse inner ear is very similar to that of humans, and observations in mice are therefore usually relevant to humans. Large mouse families, consanguineous if necessary can readily be established so that genetic linkage studies can be done. Since the mouse genome project is nearly as advanced as the human genome project, identifying and investigating candidate genes is relatively straightforward. Obtaining relevant tissues and doing physiological or other studies is normally not a problem. A number of spontaneously occurring deaf mice exist, but more recently more systematic ENU mutagenesis and screening programs have led to the creation of novel deaf mouse strains. For example, we have in collaboration with the Australian Phenomics Facility in Canberra, currently identified more than 10 new mouse strains with congenital or later-onset recessive deafness. We have identified novel "deafness" genes in some of these strains (unpublished data).

3. DEVELOPMENT AND MATURATION OF HAIR CELLS

3.1 Genes involved in hair cell differentiation

Sensorineural hearing loss - the most common type of hearing impairment - is usually accompanied by inner ear hair cell degeneration. The severity of the hearing loss is correlated to the proportion of missing hair cells. In the mature mammalian organ of Corti the hair cells are not replaced, resulting in permanent hearing impairment. However, in the avian basilar papilla (the functional equivalent of the organ of Corti) hair cells regenerate in response to cellular damage resulting in restored hearing function. This phenomenon provides hope that by understanding the mechanisms that govern the genesis and regeneration of hair cells we will be able to develop cell-based strategies to delay, prevent, or even reverse the hearing loss in individuals with hearing impairment.

There is still a limited understanding of hair cell development in mammals. The hair cells are terminally differentiated cells and the vast majority arise before birth. In the mouse, hair cell and supporting cell proliferation culminates between embryonic days 13 and 15. We know that the process is highly complex in mammals and that the Notch signalling pathway plays a central role with *Notch1* and its ligands *Delta1*, *Jagged1* and *Jagged2* expressed in the developing inner ear. Transcription factor *Math1* is also expressed in the developing ear and its presence is essential for hair cell development after hair cell precursor selection has been specified during development of the organ of Corti [14]. Hair cells are absent in mice lacking *Math1* and in contrast, the overexpression of *Math1* causes the production

of extra hair cells [15; 16]. Once hair cells have been specified, their continued differentiation requires the class IV POU-domain gene, *Pou4f3*. Without the presence of this gene product, the hair cell precursor cells degenerate.

4. FUTURE CELL-BASED THERAPIES FOR HEARING LOSS

4.1 Replacing hair cells using gene, growth factor and/or cell-based therapies

Recently there has been much interest in the role of *Math1* in hair cell differentiation and its potential use in gene therapy treatment for deafness. [15; 17]. Transfer of adenoviral vectors expressing Math1 into the ears of guinea pigs resulted in the formation of "hair cell-like" cells [18]. This group went on to show the presence of immature hair cells in the organ of Corti five weeks post-*Math1* inoculation by scanning electron micrograph analysis. At two months, the surface of the auditory epithelium contained numerous cells with mature-looking stereocilia bundles. Cross sections of the organ of Corti revealed normal appearing inner hair cells, however, in the outer hair cell area, new hair cells were poorly differentiated [19]. Therefore, other factors must be required to specify outer hair cell regeneration and there are therefore more genes to be discovered that are necessary for hair cell development and regeneration.

It has been proposed that progenitor and/or differentiated hair cells generated *in vitro* from stem cells and delivered to the inner ear might restore auditory hair cell function in the deafened mammalian cochlea. Stem cells, by definition, have a capacity for self-renewal and are able to give rise to at least one differentiated cell type. In recent years, stem cells have been identified in a wide range of mature tissues including brain, skin, muscle and blood. These stem cells are considered to be multipotent, as they are able to give rise to a limited number of differentiated cell types. In contrast, embryonic stem (ES) cells, which are derived from the inner cell mass of blastocyst embryos, are pluripotent, and therefore capable of generating all embryonic tissues including the germline.

One of the first reports of stem cell delivery to the inner ear was a study by Ito *et al* [20] that demonstrated survival and migration of adult rat neural stem cells implanted into the rat cochlea. The cells migrated to the organ of Corti, and a limited number were shown to adopt hair cell-like morphology and to stain with phalloidin. Following on from this study, a report showed that transplanted neural stem cells migrated to the vestibular epithelium and approximately 5% expressed the hair cell marker myosin VIIa after 25 days. However, only a very small number of stem cells migrated to the cochlear sensory epithelium and none of them expressed the hair cell marker myosin VIIa [21].

Since then other groups have reported on the transplantation of ES cells into the inner ear. The study by Sakamoto *et al* [22] reported survival of ES cells predominantly in the vestibular region of the mouse inner ear and also some cells in the scala media of the cochlear duct after transplantation for four weeks. In comparison, the study by Hu *et al* [23] demonstrated the survival and migration of mouse ES cells along the auditory nerve after xenotransplantation into auditory nerve fibres of the rat cochlea. We directly transplanted cells into the scala media of deafened guinea pigs and showed that these cells survived in the scala media for a post-operative period of at least nine weeks [24]. Whilst these studies have demonstrated the survival of ES cells in the cochlea, hearing function has not been restored. This would not only require that the transplanted cells survive in the cochlea, but that they integrate at the right site and become part of the highly structured organ, develop into the correct cell types, and form the right connection to nerves and other cells. This is a huge ask, and in the end it is likely that it is a combination of approaches, namely the delivery of the correctly differentiated cell type to the correct compartment of the cochlea with the right growth and neurotrophic factors that will result in the successful reversal of hearing loss.

4.2 Pharmacological treatments

Administration of glucocorticoids has in some cases shown to have a positive therapeutic effect on sudden SNHL, Meniere's disease and noise-induced hearing loss [25]. This might not only be due to the anti-inflammatory and/or immunosuppressive actions of the drugs, but also to effect of glucocorticoids on regulation of genes associated with, for example, Na^+ transport in the inner ear [26]. Knowledge about the functions of genes that are associated with hearing loss offers new opportunities for the discovery of new drugs that might prove useful in the prevention or treatment of not only congenital, but also presbyacusis and noise-induced hearing loss. For example, the discovery that many of the "deafness" genes are involved in maintaining the inner ear homeostatic salt balance would suggest that drugs that stimulate K^+ transport could be useful pharmaceuticals for the treatment of certain types of hearing loss.

The inner ear is a highly energy demanding organ and it is therefore not surprising that near-optimal mitochondrial function is a requirement for normal hearing. Hearing loss is a feature in most syndromes involving mitochondrial dysfunction. Highly toxic reactive oxygen species (ROS) are generated in the mitochondria as a consequence of energy generation. ROS damage slowly impairs mitochondrial function by damaging biological molecules (including the mitochondrial DNA) and one consequence is likely to be a progressive hearing loss [27]. Antioxidants scavenge ROS before they cause damage and slow the self-amplifying cycle of damage and increasing ROS production. Antioxidant therapy can protect hearing and hair cells from noise-induced damage and ototoxicity [28; 29].

5. CONCLUSIONS

Identification of genes associated with hearing loss has led to a much better understanding of the molecular mechanisms of hearing. Despite rapid progress in this area there are still many "deafness" genes that await identification. Our understanding of the genes that modulate noise-induced deafness, ototoxic hearing loss and the timing and severity of presbyacusis is still poor. Knowledge of the genetic factors and their contribution to hearing loss has immediate consequences for counselling

affected families and will in the future enable us to identify people at high risk of developing a hearing loss. Hopefully, we will then also have available measures for preventing or attenuating the hearing loss.

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