Chapter 30
What's the Use of Genetics?

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A.N. Popper and R.R. Fay (eds.), *Perspectives on Auditory Research*, Springer Handbook of Auditory Research 50, DOI 10.1007/978-1-4614-9102-6_30,

30.1 Why Genetics?

Genetics might seem like an obscure obsession to people who are interested in hearing loss in the human population. After all, how can a mutation causing deafness in a single family in a remote part of the world be relevant to the large numbers of people who walk through the clinic door every day complaining that they cannot hear as well as they once did? Surely everyone knows that hearing loss is caused by hair cells degenerating and once they are gone, they're gone for good? And that young people listen to music that is far too loud and that is what is damaging their hearing? And that what we need are better hearing aids? Well, yes and no. In this chapter, I hope to explain why genetics is relevant to everyone affected by hearing impairment whatever the trigger, and how genetics can be used as a first step toward developing ways of curing deafness.

30.2 Some Background—Human Deafness

Deafness is very common in the human population. Early developmental defects lead to about 1 in 850 children being born with a significant, permanent hearing impairment, and this number doubles in the first decade of life (Fortnum et al., 2001). Thereafter, progressive hearing loss increases with each decade until more than half of the population in their 70s show a significant hearing loss of 25 dB or worse (Davis, 1995). Thus, despite the clear impact of early developmental defects of the ear on individuals and their families, the vast majority of people affected by deafness suffer progressive hearing loss, so this is the major problem to be solved. Hearing loss is profoundly isolating, both socially and economically, and has a major impact on the quality of life of those affected. The only remedial options commonly available are hearing aids and cochlear implants, prosthetic devices that provide some benefits but do not restore normal function. There is an unmet need for medical approaches to slow down or reverse progressive hearing loss.

What is the role of genetics in hearing loss? Deafness is a very heterogeneous disorder, with a wide range of causes, which makes it difficult to study directly in humans. Many different genes are known to be involved in deafness. For example, for nonsyndromic human deafness, more than 130 loci have been defined and 64 genes identified (http://hereditaryhearingloss.org/), and Online Mendelian Inheritance in Man (OMIM) lists more than 800 distinct syndromes including deafness as a feature (http://www.ncbi.nlm.nih.gov/omim). More than 200 different mouse mutants with some sort of auditory system defect have been described (Steel et al., 2002; http://hearingimpairment.jax.org/index.html). Our best estimates suggest there will be at least 500 and maybe as many as 1000 genes that can be involved in deafness, any one of which can be mutated and cause deafness in an individual. Minor variations in multiple different genes (genetic background) can also interact to make a person more or less likely to develop hearing loss as they get older, and

twin, sib, and family studies have demonstrated a range of heritabilities of 0.5 or greater for age-related hearing loss indicating a significant contribution of genetics (Karlsson et al., 1997; Gates et al., 1999; Wolber et al., 2012). Noise, drugs, and infections can all contribute to hearing impairment. However, these insults do not act completely independently on hearing but instead will interact with the particular gene variants carried by an individual to influence the degree of damage. For example, the *A1555G* mutation of the human mitochondrial genome makes carriers highly susceptible to ototoxic drug-induced deafness (e.g., Estivill et al., 1998), and there are several mouse mutations that predispose the carriers to noise-induced hearing loss. Genetics (or more accurately, the assortment of genomic variants that each of us carry) is therefore an important factor in all forms of hearing impairment.

However, the vast majority of affected individuals have no molecular diagnosis. This is especially true in cases of later-onset, age-related progressive hearing loss, where we know very little about the molecular basis of the pathology. Until we have a molecular understanding of the processes underlying progressive hearing loss, it will be difficult to make progress toward developing treatments. Furthermore, improved understanding of the underlying causes of hearing loss will change the common perception of progressive hearing loss as an untreatable disorder, an inevitable part of growing older.

30.3 Genetics as a Tool

Genetics is the study of the inheritance of traits. It has two major uses for the study of deafness. First, it can be used to identify the mutations underlying hearing impairment in human families. For example, in the case of simple Mendelian inheritance in which a single gene mutation causes deafness, the trait can be linked to a chromosomal region by its co-inheritance with nearby DNA markers within the family. This will narrow down the number of genes that need to be examined for mutations that might cause the deafness. When a mutation is discovered to cause deafness in a family, this is useful in giving an explanation for the deafness and can be used to provide accurate genetic counseling to the family and better-informed prognosis of any likely future health developments, especially important for some syndromic forms of deafness. The importance to a family of knowing the cause of the deafness in their child should not be underestimated, even if there are no treatments available.

The second use of genetics is to identify molecules that are essential for normal hearing. Genetics plays a particularly important role in finding these molecules because a mutation can reveal an essential molecule even if there are only a few tens of copies in each cell (e.g., transduction channel components) and relatively few cells to analyze (e.g., inner hair cells). There are very few molecules that are present in the ear in such abundance that a biochemical or expression approach can be used for identifying them. The main examples where a biochemical approach led to discovery of an essential molecule are tectorin and prestin (Legan et al., 1997; Zheng et al., 2000), both abundant proteins in the cochlea. But these are rare exceptions,

and almost all other critical molecules have been discovered using genetics as a tool. Assembling the molecular components required for normal hearing is an important step toward building the networks of molecules that operate in auditory development and function, and these networks will offer multiple possibilities for therapeutic targets for treatments.

30.4 The Mouse as a Model

Although many genes underlying deafness have been identified directly in human families with affected individuals, it is easier to identify the responsible genes in the mouse because we can establish very large families (e.g., 100–1000 offspring) for linkage analysis to localize the mutation to a small region of a specific chromosome. Furthermore, we can minimize the number of candidate mutations to be investigated by using inbred strains of mice with defined genome sequences. More importantly, in the mouse it is possible to analyze the development of auditory dysfunction and track it back to the initial stages, which is important for determining the mechanisms involved. In contrast, by the time a human ear becomes available for detailed histological study, the pathology often has progressed to an end-state with considerable degeneration, leaving few clues to the original cause of the deafness. There are many other advantages to using the mouse to study deafness. Detailed electrophysiological measurements can be recorded in mice but not in humans, for example, endocochlear potentials in the cochlear duct or transduction currents in single hair cells in the excised organ of Corti. The mouse can be genetically manipulated to create lesions in the genome, allowing us to answer questions about the role of specific genes in auditory function. With few exceptions, mice and humans with mutations in the equivalent (orthologous) gene show similar pathologies of the ear, making them good models. Finally, if we want to understand the full range of auditory deficits in humans, we need to study a mammal. Mammals have specific features not found in other vertebrates, such as the stria vascularis generating a high endocochlear potential that provides a strong driving force across the transduction channels of hair cells, and the outer hair cells, specialized hair cells that function as amplifiers of the stimulus delivered to inner hair cells. Progressive hearing loss is quite likely to involve outer hair cell function and/or deterioration of the homeostatic state of the cochlear duct, so these two structures will be important to study.

30.5 A Long History of Deaf Mice

The earliest accounts of deaf mice date from as early as 80 BC, when "dancing" mice were described in a Chinese manuscript from the Han dynasty (Keeler, 1931). Such mice were prized by collectors because of their hyperactive, circling movements, which, we now know, were almost certainly due to balance defects. Over the

Table 30.1 Genes involved in Usher syndrome

Human type	Gene	Mouse mutant
Usher 1B	*Myo7a*	Shaker1
Usher 1C	*Ush1c*	Deaf circler
Usher 1D	*Cdh23*	Waltzer
Usher 1F	*Pcdh15*	Ames waltzer
Usher 1G	*Sans*	Jackson shaker
Usher 1J	*Cib2*	None
Usher 2A	*Ush2a*	Knockout
Usher 2C	*Vlgr1*	Frings
Usher 2D	*Whrn*	Whirler
Usher 3A	*Clrn1*	Knockout

Seven of the ten known Usher syndrome genes were found to underlie hearing and balance defects in longstanding mouse mutants

centuries, similar dancing mice were reported, until in the early 20th century some were noticed by the scientific community and taken into laboratories to breed and be studied. For example, the *shaker1* mutant was first described by Lord and Gates in 1929, and others followed over the next few decades. It quickly became apparent that these mutant mice with balance problems were most often also deaf, not surprisingly because any fundamental abnormalities in sensory hair cells or malformations of the inner ear can lead to both hearing and balance defects. Mice with severe or profound deafness can be detected by the lack of a Preyer reflex (ear flick) in response to a sudden loud sound, but the first report of electrophysiological measurements of auditory responses of a mouse mutant was published in 1940, a study of the *shaker1* mutant (Grüneberg et al., 1940).

These mouse lines carried spontaneous mutations. A background level of spontaneous mutation continues, and with so many laboratories breeding mice any obvious phenotypes such as circling that occur are likely to be noticed and either studied or passed on to other scientists interested in the type of defect revealed. Once the mutations were identified, many of these spontaneous mutants turned out to have alterations of genes that underlie Usher syndrome in humans (Table 30.1). Usher syndrome often involves balance problems as well as early deafness and later retinitis pigmentosa. The first of these genes was identified as *Myo7a* in the *shaker1* mouse mutant in a collaboration between Steve Brown and myself (Gibson et al., 1995), followed rapidly by the discovery of mutations in human *MYO7A* in people with Usher syndrome by our collaborators Christine Petit in Paris and Bill Kimberling in Omaha, Nebraska (Weil et al., 1995).

However, not all humans with hearing impairment also have balance problems. It seemed likely that other genes might be involved in causing deafness alone in the mouse, but these mutants would not be noticed in a laboratory setting as easily as mice with balance defects. This thinking motivated Professor Malkiat S. Deol at University College London in the 1950s to screen his entire mouse collection for lack of a Preyer reflex in response to a sharp, high-pitched sound. He found two lines of previously unsuspected new deaf mutants segregating within his mouse

colonies that were maintained for other purposes. These were the first two mouse mutant lines reported that showed deafness without any balance defects (Deol, 1956; Deol & Kocher, 1958). The first was called deaf (*df*, mutant line now extinct), and was an allele of the *waltzer* locus, and the second was called deafness (*dn*) and this one eventually led to the discovery of the *Tmc1* gene (Kurima et al., 2002), now thought to play a crucial role in the hair cell transduction channel. I studied for my PhD in Malkiat Deol's laboratory, and the deafness mutant was one of the first mouse mutants I worked on. During my first postdoc at the MRC Institute of Hearing Research in Nottingham, I collaborated with Greg Bock to measure cochlear function. We discovered that the deafness mutants showed no electrophysiological responses to sound stimuli at any stage (Steel & Bock, 1980), despite the presence of many intact hair cells, a subject I return to at the end of this chapter.

The mice showing hearing and balance abnormalities were initially characterized phenotypically, and this was useful in revealing a wide range of primary defects leading to deafness. However, the mutations causing these phenotypes were not discovered until much later. The identification of mutations leading to specific diseases was first reported from the mid 1980s onwards using a method called positional cloning (identifying a mutation on the basis of its position on a chromosome). One of the earliest mouse genes identified using this approach happened to be a gene involved in deafness; mutations were found in the *Kit* gene at the Dominant Spotting locus in different mouse lines with white spotting of the coat, mast cell deficiency, and deafness (Geissler et al., 1988).

In the years immediately following the emergence of the nuclear industry, research efforts were directed at studies of the biological effects of radiation and a number of new mouse mutants resulted, usually with DNA deletions, insertions, or other relatively large-scale genomic rearrangements. Several of these showed balance defects and deafness. Many of these mutations turned out to be difficult to analyze as the genomic changes were so complex, but nonetheless some have given useful insights into the role of key molecules in inner ear development. For example, we found that the Light coat and circling, *Lcc*, mutant showed local downregulation of *Sox2* and lack of sensory patch formation in the inner ear (Kiernan et al., 2005).

30.6 Chemical Mutagenesis—The Search for New Genes

As the available deaf mouse mutants were studied and the underlying genes involved identified by positional cloning, it became increasingly clear that there were far more genes involved in deafness (and other disorders) than there were mouse mutant lines. Each new mutant potentially can give access to a new molecule required for normal hearing, so we can use genetics as a tool to find the molecular components of the critical processes involved in auditory function. Therefore, efforts began to increase the number of mutant lines using chemical mutagenesis. Several programs were established using *n*-ethyl-*n*-nitrosourea (ENU), which creates point mutations (single DNA base changes) scattered around the genome. ENU is administered to

males, mutagenizing their spermatogonia, and these are mated to wild-type females a few weeks later when the active sperm carry new mutations. The offspring can be screened directly to look for new phenotypes inherited in a dominant manner, or used to build pedigrees that reveal new recessively inherited phenotypes two generations later.

From 1997 to 2000 I led a European Commission (EC)-funded program that added a screen for deafness (lack of a Preyer reflex) and balance problems (head-bobbing or circling behavior) to a wider screen of first generation offspring from ENU-mutagenized males, looking for new dominant mutations. More than 50 founder offspring with hearing or balance phenotypes were recovered at the two mutagenesis centers led by Steve Brown in Harwell, UK and Martin Hrabé de Angelis in Munich, Germany, and the mice were characterized by my team in Nottingham, Karen Avraham in Tel Aviv, and Jean-Louis Guénet in Paris. Over the past 10 years more than half of these mutations have been identified by positional cloning by the five groups in the consortium. Some mutant lines had mutations in known deafness genes, and some loci showed multiple independent mutations possibly reflecting ascertainment bias due to a strong circling or head-bobbing phenotype (e.g., *Chd7* was mutated in multiple independent lines; Bosman et al., 2005), but altogether 10 new genes were identified as underlying hearing and balance defects through this program.

We learned some useful lessons from the hearing and balance screens as part of the ENU mutagenesis programs. First, there was a very wide range of phenotypes found, ranging from ossicle malformations through semicircular canal truncations to hair bundle orientation anomalies. Second, many of the phenotypes were not obvious without targeted screening, particularly hearing impairment, and several of the new mutations found were in genes that had already been knocked out and published but with no mention of any hearing or balance problem. Thus, as a general rule, we find only what we look for. Third, if we had started with the full list of 20,000 mouse genes, there was no prior evidence that would have led us to guess that these ten new genes would be required for normal hearing, suggesting that it is difficult to predict which genes are involved in deafness based on our current incomplete knowledge. Overall, the phenotype-driven approach (starting with a deaf mouse and finding the causative mutation) appeared to be a valuable way of identifying new genes involved in deafness and new pathological mechanisms.

The key to success for the large-scale ENU mutagenesis programs was close engagement between the screening teams and the groups analyzing the variant lines after initial discovery. Our program was a success because both screeners and experts in hearing and balance were funded by the same EC program facilitating close interaction. It was the experts in hearing and balance function who devised the screens and took on the resulting new mutant lines to study, while the experts in ENU mutagenesis produced the large numbers of mice with an optimum mutation density to screen. However, this success was not the case for all ENU screens, and researchers were sometimes disappointed at the difficulty in reproducing the initial phenotype in their own laboratory and were daunted by the prospect of having to identify the underlying mutations by positional cloning, which can be

time-consuming with no guarantee of success. Furthermore, the benefits to be gained from ENU mutagenesis can be long term; for example, one of the last of the deaf mutants recovered from our 1997–2000 screen was published more than 10 years later, after exome sequencing became feasible (Hilton et al., 2011). High expectations of rapid progress were not fully realized and many ENU programs were funded for only a single grant cycle.

30.7 Targeted Mutagenesis

More recently, targeted disruption of specific genes has become the focus of attention. The advantage of this approach is that it should not be necessary to identify the gene by positional cloning, a significant shortcut. The first gene to be "knocked out" by genetic manipulation in embryonic stem (ES) cells that were then used to create a new mutant mouse line was a gene affecting inner ear development, *Fgf3*, previously known as *Int2* (Mansour et al., 1993). Since then, more than 6000 genes have been knocked out and reported (MGI; http://www.informatics.jax.org/). These have been generated in multiple laboratories, using different targeting strategies and various, often mixed, genetic backgrounds. Also, they are often not made available to other researchers.

Inspired by the success of targeting as a strategy and the drawbacks of the existing resource, an ongoing international effort (KOMP, EUCOMM) has resulted in more than 13,000 mouse genes being targeted in ES cells on a single inbred genetic background (C57BL/6N) and made available to all researchers from public repositories (Skarnes et al., 2011). When I moved from Nottingham to the Wellcome Trust Sanger Institute in 2003, I took advantage of the growing ES cell resource to establish a new screen, the Mouse Genetics Project, using these targeted ES cells to generate mice to screen for a wide range of phenotypes, including hearing impairment. Instead of using the Preyer reflex, which can detect only severe or profound deafness, we developed a rapid auditory brain stem response (ABR) protocol that takes only 15 minutes to perform and can detect mild and moderate hearing impairments too (Ingham et al., 2011). So far we have generated more than 800 new mutant lines and screened more than 600 of these by ABR. We have found 12 new genes associated with raised ABR thresholds, plus a number of further lines where we see normal thresholds but robust anomalies of the ABR waveform, suggesting that these mice may have a central auditory system processing defect.

Just as for the ENU screen, none of these genes was previously suspected of being involved in auditory function. The hearing impairment ranges widely, from mild or moderate threshold increases, to high-frequency hearing loss or severe deafness across all frequencies. Waveform anomalies are likewise varied, including some with small amplitudes of early waves but normal or near-normal later waves, and others with prolonged latencies. The underlying pathologies include middle ear inflammation, synaptic abnormalities and reduced endocochlear potential. Of these 12 new mutant lines with hearing impairment, only one would have been detected

using the Preyer reflex, emphasising the benefit of using ABR to screen. The data from this screen can be viewed on the Sanger Institute website (http://www.sanger.ac.uk/mouseportal/) and all mutant lines are made available to the scientific community through public repositories (http://www.findmice.org/index.jsp).

30.8 What Have We Learned from Deaf Mouse Mutants?

Clearly we have learned a great deal about the role of many individual genes in auditory function over the past 20 years and more, but what general lessons have we learned from mice that are relevant to human deafness? Study of the mouse has led us to a number of key observations that really could not be deduced from study of human hearing alone, and I have listed a few below.

The fact that so many completely unexpected genes have been found by systematic screening for hearing impairment by ABR (12 with raised thresholds out of the first 600 mutant lines screened) suggests that there are many genes required for normal hearing waiting to be discovered and that there will probably be well over 500 genes associated with deafness. This suggestion is supported by the limited overlap in genes currently known to be involved in deafness in mice and humans (Fig. 30.1). Recently discovered genes lie mostly outside the region of overlap, because it takes time for a knockout mouse to be generated after a gene has been discovered in humans, and equally it takes time for a human family to be found with a mutation of a candidate gene identified in the mouse. Ultimately the gene sets will merge and then we will know we are close to finding all the genes associated with hearing impairment.

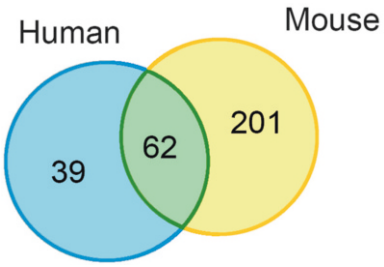

Fig. 30.1 Human and mouse deafness genes. The number of genes so far known to be associated with deafness in mice and humans. With very few exceptions, genes found associated with deafness in the mouse are eventually found in humans with deafness, and genes underlying human deafness are usually knocked out in the mouse, leading to deafness. The limited overlap reflects the rapid progress in recent years in identifying genes involved in deafness in both species, and when we approach the complete ascertainment of deafness genes the two circles are expected to merge. Genes included are those listed in the Hereditary Hearing Impairment in Mice website maintained by Ken Johnson at the Jackson Laboratory (http://hearingimpairment.jax.org/index.html) and the Hereditary Hearing Loss Homepage maintained by Guy Van Camp and Richard Smith (http://hereditaryhearingloss.org/)

Furthermore, more than 800 distinct human syndromes involving hearing impairment have been catalogued in OMIM, indicating that 500 may be an underestimate.

Access to tissues from normal mice at various developmental stages has been valuable for expression studies. The distribution of mRNA or protein derived from genes involved in deafness has demonstrated expression in many different locations in the auditory system, such as the stria vascularis and lateral cochlear wall, supporting cells, neurons, tectorial membrane, or middle ear epithelium, as well as in sensory hair cells, showing that the function of the entire auditory system is important for hearing.

Very few mouse mutants published so far have been shown to have deafness with a central auditory system origin and with a normally functioning cochlea, suggesting that most deafness is sensory rather than neural. However, as mentioned earlier, in our large-scale screen (the Mouse Genetics Project), we have observed a number of mutant lines with normal ABR thresholds but abnormal waveforms indicating a problem with central processing. Maybe we have not found many mice with central auditory defects because previously we focused on raised thresholds and so have not captured other anomalies.

We have found a very wide range of defects leading to hearing impairment in the mouse mutants we have studied. Although we can distinguish conductive from sensorineural hearing loss and sensory dysfunction from auditory neuropathy using standard audiological methods in humans, the wide range of pathologies found in the mouse indicates that better diagnostic methods will be critical to the choice of treatments when these are available in the future. For example, there will be no point in attempting to regenerate sensory hair cells that will not function due to an inherent defect in the hair cell itself or to dysfunction elsewhere in the cochlear duct.

Analysis of the time course of pathological events in the mouse indicates that although hair cells are often the earliest cell type to degenerate in the cochlea, in due course the surrounding supporting cells also degenerate. As these have key roles in hearing, any treatments involving stimulating regeneration will need to regenerate the whole sensory patch and not just hair cells.

Finally, hair cell degeneration is an extremely common feature in mouse mutants and humans with hearing impairment, and of course once a hair cell has degenerated it can no longer function. However, out of more than 100 different mouse mutant lines I have studied in my own laboratory, in no case was the hair cell loss the primary cause of the deafness. In every case, there was some form of damage or dysfunction of hair cells before they degenerated, suggesting that degeneration is a secondary effect following dysfunction, an epiphenomenon. There are many mutants in which for the first few weeks of life there is a complete set of hair cells present but no auditory responses can be obtained. It is not clear why dysfunction leads to hair cell death, but it seems to be a universal consequence. Detailed studies of noise-exposed cochleas also indicate that it is damage and not degeneration that corresponds most closely to threshold shifts (Liberman & Dodds, 1984). This observation is not clear from studying human pathology alone, because most inner ear samples from humans come from people who have suffered deafness for many years and the cochlea will be at an end-stage of the pathological process leaving few clues to the initial causes of hearing loss.

30.9 Goals for the Future

Assembling the molecular components supporting normal auditory function will be an important goal, and genetics will continue to be a key tool in identifying those components. We can think about hearing as a 1000-piece jigsaw puzzle; we are making some progress with putting together small sections of the puzzle by identifying clusters of interacting molecules but having the complete set of pieces will enable us to see the full picture. Much current research is focused on a well-known set of genes, but the picture will be complete only when light is shone on the total set of genes involved (Fig. 30.2; Edwards et al., 2011).

Generating and screening mouse mutations representing all 22,000 mouse genes is the long-term goal of the International Mouse Phenotyping Consortium (IMPC; Brown & Moore, 2012), and the success of the ABR screen within the Sanger Institute's Mouse Genetics Project has led to the adoption of ABR as a standard screen by the IMPC. This will be a major contribution to finding more genes required for normal hearing and candidates for human deafness.

The IMPC is currently using the EUCOMM/KOMP targeted ES cell resource as a source of the mutant lines to be screened. However, not all genes are targeted in

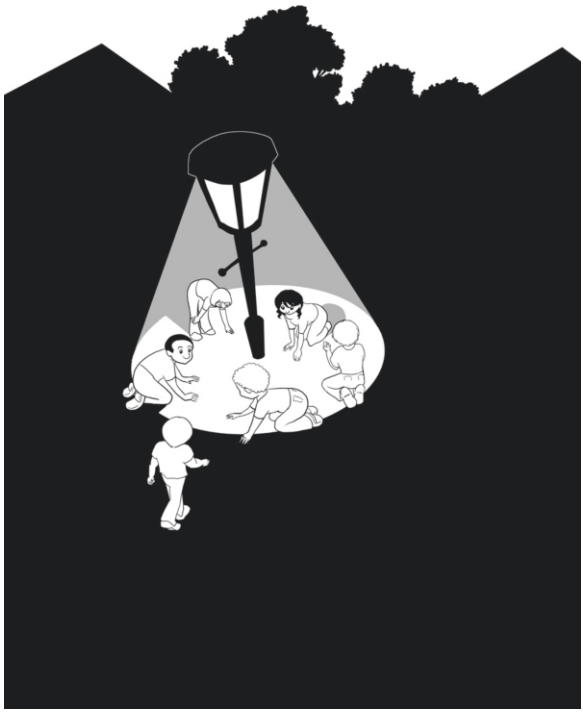

Fig. 30.2 Looking under the lamppost. We all spend time looking at our favorite genes that are well-studied and have good resources available, but it is important not to forget the dark matter outside the beam of light—the many genes that have not yet been identified as being involved in hearing

this library and the last 20 % may be particularly difficult to target for an assortment of technical reasons. At that point, it seems that a return to ENU as a mutagen will be useful, but using ENU in a gene-driven way rather than the phenotype-driven screens previously used. Several groups are building up libraries of DNA samples from thousands of male offspring of ENU-treated mice together with associated frozen sperm that can be used to recover living mice by in vitro fertilization techniques. The rapidly reducing cost of DNA sequencing has facilitated sequencing of the coding regions of the genome (the exome) from these samples, and mutations are detected, assessed, and displayed using bioinformatic tools. Thus it is a relatively simple matter to select a suitably damaging mutation of the gene of interest and order the resurrection of the line from the corresponding sperm sample. It is likely that this approach will enable the completion of the production of a set of mutant mouse lines representing all known mouse genes, and probably many nonprotein–coding elements like microRNAs and long noncoding RNAs as well. The use of these ENU resources will be valuable also for producing allelic series of different mutations of the same gene to confirm association of the phenotype with the gene and explore the role of specific sequence motifs in the function of the molecule.

A complete set of genes associated with deafness in the mouse will be a valuable aid to interpreting exome sequence in people with deafness. Every person carries a huge number of DNA variants (around 3 million) including potentially pathogenic mutations in many genes. Having a shortlist of likely candidate genes from the mouse will help reduce the number of variants that require further study to a more manageable level, even if that shortlist contains a thousand genes. The mouse can provide added confidence in the association between the phenotype and the sequence variant and hence support accurate diagnosis in humans. This will be particularly useful for cases of syndromic deafness.

However, it seems most unlikely that the cause of human nonsyndromic deafness could be diagnosed by genome analysis alone. Linkage analysis will help if the inheritance of deafness can be tracked in a family with linked DNA markers that can indicate which part of which chromosome contains the causative mutation, but often this will still result in a very large number of genes to consider. We need to know considerably more about the genes associated with deafness and the pathogenicity of specific types of mutation before sequence data in individuals could be used for diagnosis without other supporting evidence. For this reason, I envisage that further development of other diagnostic tools using audiological, psychoacoustic, electrophysiological, and imaging approaches would be a valuable complement to sequence analysis and point toward an underlying mechanism in each person before more sophisticated treatments can be applied. Well-characterized mouse mutants with different pathologies can be useful for linking known primary mechanisms with measurable features that can be transferred to clinical use. One key differential diagnosis required will be distinguishing a primary sensory hair cell defect from a problem in maintaining homeostasis of the cochlear fluids, as these types of deafness will need quite different approaches to treatments.

What about development of medical treatments for hearing loss? Despite the extreme heterogeneity of causes of deafness, there are several good reasons to

believe that treatments are an achievable goal. First, a large proportion of people affected by deafness show progressive hearing loss, and it is much easier to imagine stopping or reversing the progressive deterioration of a system that once worked well than to find treatments for early developmental defects. Even slowing down the rate of progression of hearing loss would be useful. Second, the target population is considerable and getting larger as people live longer, making it more likely that large pharmaceutical companies will see development of treatments as a worthwhile investment. Third, it is likely that many different primary causes for hearing loss, such as mutations in different genes or responses to different environmental insults, may operate within a limited set of networks of molecular interactions. There may be common points within each network that could be targeted by small molecule or other interventions, meaning that people with different primary causes for deafness could be grouped together and benefit from a common treatment. Finally, there are already some forms of treatments that have been shown to be useful in animal models and as we understand more about new forms of deafness using the mouse, these opportunities are likely to increase. Some mechanisms we are studying in mice are known to be amenable to small molecule manipulation, such as systemic immune or cardiovascular diseases.

As we move closer to a complete catalogue of genes/molecules required for auditory function we will be able to explore the functional relationships between these molecules in pathways and networks, and then focus on those molecules/pathways/networks that are of greatest importance to the human population. The identification of these pathways through the use of genetics will be relevant to all causes of hearing impairment including those with a primary environmental trigger. Drawing up a preliminary network of gene interactions is a straightforward process using bioinformatic tools and available databases that utilize a wide range of sources of information. However, ensuring that each interaction (or edge) is relevant to the auditory system requires detailed follow-up to ask if the components are expressed in the relevant cell type (e.g., in the hair cell) and if the nature of the interaction (e.g., up- or downregulation) is supported by experimental evidence. This is not a simple task because such interactions between each pair of molecules may vary depending on the context—which cell type and which time of development is studied.

Despite the complications of building networks, these will prove to be an invaluable resource for supporting drug development. For example, some key molecules in the network may already have approved small molecules used for other disease indications, and repurposing is likely to be an important activity to ensure the maximum benefit is gained by both the patient and the organization that invested in development of each drug.

Building networks has another valuable purpose—identifying molecules that may play a critical role in hearing but also are essential for survival. These molecules would not be detected by a program that focuses on knocking out the function of the gene because there would be no offspring surviving to test for deafness. In our ABR screen of new mouse mutants described earlier, we screen heterozygotes in cases where the homozygote is lethal. The phenotypes we detect in heterozygotes

suggest that knocking down the level of protein production has an impact on cellular function. Of course, mutations in humans are extremely variable and include gain-of-function as well as loss-of-function effects, so the system will be complex. Building networks that tap into the entire existing data set of molecular interactions can overcome the gaps that exist if only genes underlying deafness are considered. Thus, networks can generate hypotheses to be tested, for example, by using conditional knockouts affecting only the ear or relevant part of the auditory system.

Furthermore, construction of networks can point to genes that are redundant in auditory function. In these cases, knocking out the gene will not lead to deafness because an alternative gene can operate in its place. In normal circumstances, a level of redundancy leads to a more robust system. However, it may be that when the organism is put under stress, such as exposure to noise, the alternative gene alone is not as efficient at resisting the damaging effects as both genes together would be. Therefore, many of the genes that so far appear to have no obvious role might be required when the individual is exposed to damaging environments. Networks and pathways can reveal the redundant molecules operating between the nonredundant molecules known to be associated with deafness, and thus open up a broader range of targets for development of therapies.

Finally, I return to a question that has puzzled me since I first started working on deafness. Why do hair cells die? For part of my PhD, I studied three mutants (*deafness*, *jerker*, and *varitint-waddler*) plus mice treated with an antithyroid agent to produce hypothyroidism. All showed progressive degeneration of hair cells but had no responses to sound at a stage when most hair cells were still present, as mentioned previously in this chapter. The hair cells were clearly present and not functioning properly, but why did this lead to their death? Does the lack of normal function lead to a disruption in their cellular homoeostatic mechanisms, which must be highly adapted to manage a continuous flow of cations during transduction? Does the lack of normal synaptic activity lead to loss of a putative trophic role of auditory neurons? Or is there a loss of the normal function of supporting cells to support hair cell survival following abnormal hair cell activity? Although we now know the three mutations underlying deafness in these three mutants, I am still not sure we understand the reason for the hair cell death. There was a clue, however, in the pattern of hair cell loss common to all of them. The earliest signs of loss were a few scattered hair cells mostly in the basal half of the cochlear duct and over a few days this scattered loss extended towards the apical turn. Then, superimposed on the scattered pattern of loss there appeared patches where all hair cells as well as some of the supporting cells appeared to have degenerated. I wondered at the time if there was a tipping point where the loss of a single hair cell could be managed but if two or three hair cells close to each other died this led to a more widespread loss of homeostasis within the organ of Corti and a whole patch would degenerate rapidly. If this is the case, then are hair cells releasing a trophic agent that sustains the health of adjacent cells? The reason why hair cells die remains one of the key questions in auditory research, because interfering with that process might give us insights into how to preserve hair cells into old age.

Acknowledgments I thank Morag Lewis for drawing the figures and commenting on the manuscript and my many colleagues and collaborators who have helped me develop the thoughts presented in this essay. The work was supported by the Wellcome Trust, the MRC, and the EC.

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