Mouse Models for Deafness: Lessons for the Human Inner Ear and Hearing Loss

Karen B. Avraham

In the field of hearing research, recent advances using the mouse as a model for human hearing loss have brought exciting insights into the molecular pathways that lead to normal hearing, and into the mechanisms that are disrupted once a mutation occurs in one of the critical genes. Inaccessible for most procedures other than high-resolution computed tomography (CT) scanning or invasive surgery, most studies on the ear in humans can only be performed postmortem. A major goal in hearing research is to gain a full understanding of how a sound is heard at the molecular level, so that diagnostic and eventually therapeutic interventions can be developed that can treat the diseased inner ear before permanent damage has occurred, such as hair cell loss. The mouse, with its advantages of short gestation time, ease of selective matings, and similarity of the genome and inner ear to humans, is truly a remarkable resource for attaining this goal and investigating the intrigues of the human ear.

(Ear & Hearing 2003;24;332–341)

The Mouse in Biomedical and Inner Ear Research

The mouse has proven itself time and again as an exemplary model system for mammalian biology and human disease. This furry creature, though offensive to some, has provided insight about many human diseases and disorders, including cancer, heart disease, neurological disease, and most notably, hearing impairment. There is already an impressive collection of mouse mutants with hearing and/or vestibular dysfunction. In 1980, before there was discussion of the sequencing of the mouse genome (or human genome), there were but 50 mutant genes known to cause inner ear defects in mice, based on existing deaf mouse mutants (Deol, 1980). Today, not only have many of these mutant genes been cloned, but there are more than 80 mutants generated by gene-targeted mutagenesis (knockouts) alone with an inner ear phenotype (Anagnostopoulos, 2002) (Table 1).

Mice provide several advantages over other model organisms, particularly for the field of hearing research. First, the mouse is a mammal and hence its cochlea is remarkably similar to that of humans, despite other clearly observable differences between the two species. As in humans, the mechanosensory cells in mice are responsible for detecting sound in the cochlea and gravity and acceleration in the vestibular system. In the mouse organ of Corti, hair cells are arranged in one row of inner hair cells (IHC) and three rows of outer hair cells (OHC), with actin-rich stereocilia projecting on their apical surface. In the vestibular system, hair cells are arranged in patches in the saccule, utricle and semicircular canals. Defects in the vestibular system, often associated with deafness, are more severe in mice, leading to head bobbing or circling. For this reason, deaf mice have been easily recognizable; in fact, until a few years ago, the only mouse mutant involving the ear without vestibular dysfunction was the deafness (*dn*) mouse (Steel & Bock, 1980).

For experimental procedures, the mouse has long been recognized as a model of choice since its gestation time of 3 wk is relatively short. With proper care, mice thrive under controlled laboratory conditions. A major advantage offered by mice is the availability of inbred strains—genetically identical or isogenic mice—that provide a standard of comparison when comparing experiments between different laboratories. They also enable the study of a parameter without the complication of variable genetic background when repeating experiments. To become isogenic, inbred strains are generated after 20 generations of brother-sister matings (Silver, 1995; www.informatics.jax.org/silver/). Common inbred lines are C57Bl/6 (for which a draft genome sequence is now available; see below), BALB/c, and 129/Sv. More specialized strains, such as congenic (mice with a chromosomal segment derived from one strain on the background of another inbred strain) and consomic/chromosome substitution strains (CSSs; entire chromosome placed on different genetic background) continue to play a major role in mouse genetics, particularly in identifying quantitative trait loci that underlie polygenic disease (Nadeau, Singer, Matin, & Lander, 2000).

Excellent web resources are available that deal with mouse models for human disease and disorders, with some covering the inner ear in particular (Table 1).

Department of Human Genetics and Molecular Medicine, Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel.

DOI: 10.1097/01.AUD.0000079840.96472.DB

0196/0202/03/2404-0332/0 • Ear & Hearing • Copyright © 2003 by Lippincott Williams & Wilkins • Printed in the U.S.A.

TABLE 1. Web resources for mouse models for deafness

Resource	URL	Source
Hereditary Hearing Impairment in Mice	www.jax.org/research/hhim	The Jackson Laboratory
List of mouse inner ear mutants	www.ihr.mrc.ac.uk/hereditary/MutantsTable.shtml	MRC Institute of Hearing Research
NIDCD Mouse Organ of Corti Library	http://neibank.nei.nih.gov/Ear/NbEar.shtml	National Eye Institute-National Institutes of Health
A compendium of mouse knockouts with inner ear defects	http://tbase.jax.org/docs/inner-ear-ko-compendium.html	The Jackson Laboratory

The History of the Mouse as a Model Organism

Human and mouse lineages are thought to have diverged between 90 and 140 million years ago (Bromham, Phillips, & Penny, 1999). The first records of mouse mutants referred to coat color and other easily recognizable phenotypes, most notably dominant-spotting, albino, waltzing, and yellow mice. Referred to as "mouse fanciers," collectors in Japan, China and Europe in the 18th and 19th century bred these mice, generating what would later become laboratory mice. Once Mendel was rediscovered, pioneers in mouse genetics duplicated Mendel's pea experiments in mice, leading to the creation of inbred mice, many of which are used today (Silver, 1995). The first report of genetic linkage in the mouse was that of the pink-eye dilution and albino loci, based on mouse crosses between mouse mutants with these phenotypes. More than half a decade later, the genes on chromosome 7 associated with these phenotypes were identified (Gardner et al., 1992; Kwon, Haq, Pomerantz, & Halaban, 1987). In 1982, there were 45 loci on chromosome 7, representing mouse mutants, and serological and enzyme polymorphisms. Recombinant DNA technology provided the tools that led to where we stand today, with chromosome 7 represented by ~2645 protein-encoding genes (Mouse Genome Resources, NCBI; www.ncbi.nlm.nih.gov/genome/guide/mouse/index.html) and 122 Mb in sequence (Waterston et al., 2002). In some cases, it took the completion of the draft sequence to identify genes not previously identified in one or the other genome. For example, otoferlin was discovered to be associated with human deafness in 1999 (Yasunaga et al., 1999), but it was only with the sequencing of the mouse genome that the mouse orthologue was found (Waterston et al., 2002).

Mouse genetics has a long history, much before the structure of chromosomes was even known. The early 1900s brought recognition of the mouse as a model for human disease, and interest in these animals shifted from the mouse fanciers to the laboratory. As early as 1928, spontaneous and radiation-induced mutations were discovered to have hearing and vestibular defects, for example, shaker 2 (*sh2*) (Dobrovolskaia-Zavasdkaia, 1928), but it was not until the last decade of the 20th century that the gene responsible for the phenotype was discovered (Probst et al., 1998).

The Mouse Genome

Comparative genomics has provided an incredibly useful tool for identifying human disease genes. Stretches of 1 to 50 centimorgans of chromosomes are conserved between humans and mice, with orthologous genes present along each chromosome. In fact, there are approximately 200 such shared homologous regions between human and mouse chromosomes, termed "syntenic" regions (Copeland, Jenkins, & O'Brien, 2002). Existing mouse models for some of these genes have allowed predictions of candidate genes for human disease and disorders. One of the truly remarkable advances for using the mouse as a model for human disease is the ability to determine the chromosomal localization of a mouse gene and correlate it with the human chromosomal location and when available, mouse mutant. The field of hearing loss has demonstrated the value of comparative genomics over and over again. For example, the *Pou4f3* mouse gene location and mouse knockout provided the key for discovering the gene for a human form of non-syndromic progressive hearing loss, DFNA15 (Vahava et al., 1998; Van Laer, Cryns, Smith, & Van Camp, 2003). Identification of the mouse gene for spinner (*sr*), shaker 1 (*sh1*), and shaker 2 (*sh2*) led to the identification of the orthologous human genes and human deafness loci for DFNB6, USH1B/DFNB2/DFNA11, and DFNB3, respectively (see Table 2). The opposite holds true as well; in some cases, discovery of the human deafness genes has led to identification of the mouse genes, as was the case for DFNB7/B11 and DFNA36 for the genes for deafness (*dn*) and Beethoven (*Bth*), respectively (see Table 2).

Initial sequencing of the mouse genome has revealed a tremendous amount of valuable information for biomedical research. The draft sequence of the human genome, released in 2001 (Lander et al., 2001), was followed by that of the mouse in late 2002 (Waterston et al., 2002). The

TABLE 2. Genes for which both a human and mouse form of deafness exists

Human Locus	Human Gene	Protein	Mouse Mutant	No. Alleles[1]	Reference for Mouse Mutant/Gene Cloning
Non-syndromic hearing loss					
DFN3	*POU3F4*	Pou3f4	Sex-linked fidget (*Slf*); *Pou3f4* knock-outs	3	Minowa et al., 1999; Phippard, Lu, Lee, Saunders, & Crenshaw, 1999)
DFNB1	*GJB2*	Connexin 26	Conditional knock-out ($Cx26^{OtogCre}$)	2	(Cohen-Salmon et al., 2002)
DFNB2/DFNA11	*MYO7A*	Myosin VIIa	shaker 1 (*sh1*)	9	(Gibson et al., 1995)
DFNB3	*MYO15A*	Myosin XVa	shaker 2 (*sh2*)	3	(Probst et al., 1998)
DFNB6	*TMIE*	Transmembrane inner ear (Tmie)	spinner (*sr*)	2	(Mitchem et al., 2002)
DFNB7/B11/A36	*TMC1*	Transmembrane, cochlear expressed 1	deafness (*dn*), Beethoven (*Bth*)	2	(Kurima et al., 2002; Vreugde et al., 2002)
DFNB12	*CDH23*	Cadherin 23	waltzer (*v*)	10	(Di Palma et al., 2001; Wilson et al., 2001)
DFNB36	*ESPN*	Espin	jerker (*je*)	1	(Naz, Riazuddin, Riazuddin, Griffith, Friedman, & Wilcox, 2002)
DFNB37/A22	*MYO6*	Myosin VI	Snell's waltzer (*sv*)	2[2]	(Avraham et al., 1995)
DFNA15	*POU4F3*	Pou4f3	*Pou4f3* knock-outs; dreidel (*ddl*)	3	(Erkman et al., 1996; Xiang et al., 1997)
Syndromic hearing loss					
Alport syndrome	*COL4A3*	Procollagen, type IV, •3	*Col4a3* knock-out	3	(Cosgrove et al., 1996)
BOR syndrome	*EYA1*	Eyes absent 1 homolog	*Eya1* spontaneous mutation; knock-out	2	(Johnson et al., 1999; Xu, Adams, Peters, Brown, Heaney, & Maas, 1999)
Jervell & Lange-Nielsen Syndrome 1 (JLNS1)	*KVLQT1*	Kvlqt1	*Kvlqt1* knock-out	5	(Lee et al., 2000)
Jervell & Lange-Nielsen Syndrome 2 (JLNS2)	*KCNE1 (ISK)*	Kcne1 (Isk)	*Isk* knock-out; punk rocker (*pkr*)	2	(Vetter et al., 1996)
Norrie disease	*NDPH*	Norrin	*Ndph* knock-out	1	(Berger et al., 1996)
Pendred syndrome	*SLC26A4 (PDS)*	Solute carrier family 26, member 4 (pendrin)	*Slc26a4* knock-out	1	(Everett et al., 2001)
Stickler syndrome	*COL11A2*	Procollagen, type XI, •2	Col11a2 knock-out	1	(McGuirt et al., 1999)
Usher syndrome type IB[2]	*MYO7A*	Myosin VIIa	shaker 1 (*sh1*)	9	(Gibson et al., 1995)
Usher syndrome type 1D (USH1D)	*CDH23*	Cadherin 23	waltzer (*v*)	10	(Di Palma et al., 2001; Wilson et al., 2001)
Usher syndrome type 1F (USH1F)	*PCDH15*	Protocadherin 15	Ames waltzer (*av*)	6	(Alagramam, Murcia, Kwon, Pawlowski, Wright, & Woychik, 2001)
Waardenburg syndrome type 1 and 3 (WS1, WS3)	*PAX3*	Pax3	Splotch (*Sp*)	15	(Epstein, Vekemans, & Gros, 1991)
Waardenburg syndrome (WS2)	*MITF; SLUGH*	microphthalmia-associated transcription factor; SLUG zinc finger	microphthalmia (*mi*); *Slugh*	21	(Hodgkinson et al., 1993); (Perez-Losada et al., 2002)

[1] According to Mouse Genome Informatics at the Jackson Laboratory http://www.informatics.jax.org/. These include knock-outs, spontaneous, radiation- and chemical-induced mutations.
[2] se^{sv} is also a Myo6 allele; not reported at Mouse Genome Informatics.

draft sequence, covering 96% of the euchromatic genome of the commonly used C57Bl/6J strain of mice, is approximately 14% smaller than the human genome. Both genomes, however, contain about 30,000 genes and less than 1% of mouse genes have no human orthologue. In fact, encoded proteins between mouse and human have a median amino acid identity of 78.5%. This remarkable genetic and protein similarity provides the basis for comparative study between mice and humans.

A Plethora of Mouse Mutants

There are three major forms of mouse mutants—spontaneous, radiation or chemical induced, and transgenics or knock-outs. For hearing and vestibular mutants, the first form numbers approximately 60 that have arisen spontaneously in mouse facilities throughout the world over many years. Many of these are derivatives of the mouse fanciers collections. Examples of these mutants are shaker 2 (*sh2*), shaker 1 (*sh1*), waltzer

(*v*), and Snell's waltzer (*sv*), first described in 1928, 1929, 1945 and 1966, respectively (Deol & Green, 1966; Dobrovolskaia-Zavasdkaia, 1928; Gates, 1929; Snell, 1945). All are now known to be models for human forms of syndromic and nonsyndromic deafness, and in several cases, the mouse deafness gene led to the discovery of the human deafness gene. For example, a myosin VI mutation causing deafness in the Snell's waltzer mouse led investigators to specifically search for human mutations in this gene. Human myosin VI mutations are now known to be associated with both dominant and recessive forms of hearing loss (Ahmed et al., 2003; Melchionda et al., 2001)

The second major form of mouse mutants are radiation or chemical induced, and in some cases were byproducts of large-scale mutagenesis experiments designed to study the effects of radiation on the germline (Russell, 1971). Notable examples of radiation-induced mutations are alleles of piebald with mutations in *Ednrb*, a model for Waardenburg syndrome type IV (WS4; Hirschsprung disease) (Shin, Russell, & Tilghman, 1997), the short ear Snell's waltzer (se^{sv}) allele of the deaf Snell's waltzer (Avraham et al., 1995), and the first allele of shaker 2 (*sh2*) (Dobrovolskaia-Zavasdkaia, 1928). Mouse spermatogenic cells are particularly susceptible to mutagenesis by chemicals such as N-ethyl-N-nitrosourea (ENU) and chlorambucil (CHL). Most recently, several large ENU-mutagenesis screens have been initiated (Justice, Noveroske, Weber, Zheng, & Bradley, 1999), leading to a large number of mouse models for human diseases including hearing and balance mutants, including a model for otitis media in children (Hardisty et al., 2002; Kiernan et al., 2001; Tsai et al., 2001; Vreugde et al., 2002).

The third and most "modern" of the mouse mutants are transgenics and gene-targeted knock-outs. The first transgenic mouse made by microinjection of a foreign gene directly into the embryo was in 1980 (Gordon, Scangos, Plotkin, Barbosa, & Ruddle, 1980). Since then, the advances in this area have been dramatic, with the ability today to conditionally knock-out a gene in a specific tissue (Kuhn, Schwenk, Aguet, & Rajewsky, 1995), as was done to study the loss of connexin 26 specifically in the inner ear of mice (see below; Cohen-Salmon et al., 2002). Standard transgenic mice typically contain 1 to 1000 copies of the microinjected foreign gene, with the gene usually up to 10 kb in length. This requires the gene to be injected to be small, or to inject the cDNA, in which case expression can often be compromised due to lack of introns. Bacterial artificial chromosomes (BACs) containing foreign genes of up to 300 kb in length (Shizuya et al., 1992) can now be injected to produce transgenic mice (Yang, Model, & Heintz, 1997). BAC transgenesis was used to rescue the *sh2* circling and deaf phenotype, leading to the discovery of the myosin XVa (*Myo15a*) gene for both the *sh2* phenotype and a recessive form of nonsyndromic deafness, DFNB3 (Probst et al., 1998; Wang et al., 1998).

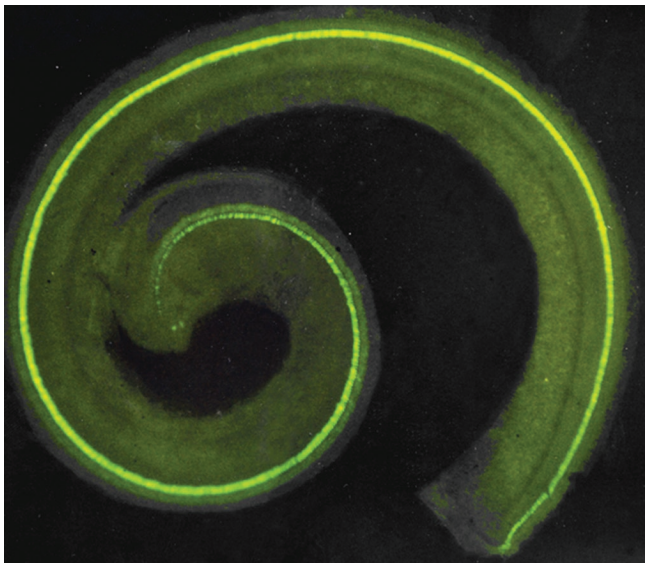

Figure 1. The GFP reporter is used to visualize expression of myosin VIIa in the inner and outer hair cells of a P2 mouse cochlea, driven by the newly identified promoter. Reprinted with permission from *Human Molecular Genetics*.

Transgenesis offers the advantage of allowing one to follow expression of a gene using a reporter gene, such as green fluorescent protein (GFP) or the LacZ/●β-galactosidase gene. To identify the myosin VIIA promoter, the GFP reporter gene was subcloned under the control of several putative promoter regions of myosin VIIA and injected into mouse embryos to generate transgenic mice (Boeda, Weil, & Petit, 2001). The inner hair cells and outer hair cells of the apical turn were labeled green when exposed to fluorescence, corresponding to regulation of expression of myosin VIIa in these cells (Fig. 1).

Gene-targeted mutagenesis, or knock-out technology, is an extension of transgenic technology, whereby a mutation is made in embryonic stem cells, and once cells carrying this deletion or insertion are selected, they are injected into blastocysts that are implanted into mice (Joyner, 2000). Endogenous gene expression can be studied by substituting the gene with a reporter gene with gene-targeted technology and observing reporter gene expression in the heterozygous state. Expression of the *Math1* gene was visualized by replacing the *Math1* coding region with β-galactosidase, showing localization of this gene in the sensory epithelium of the cochlea

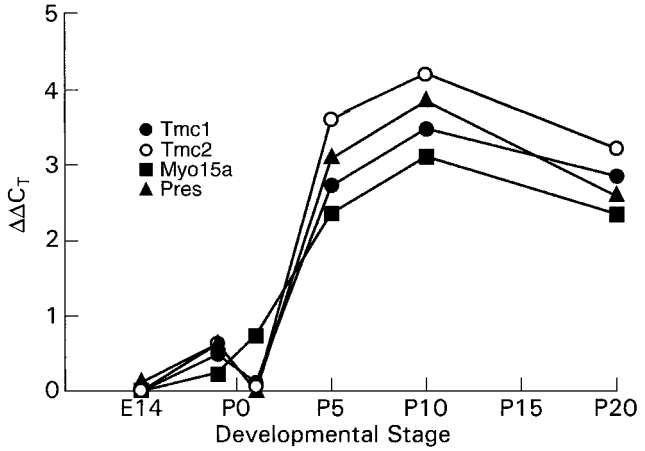

Figure 2. Real-time RT-PCR analysis of *Tmc1, Tmc2*, myosin XVa (*Myo15a*), and prestin (*Pres*) mRNA levels in mouse temporal bones at E14, E18, P1, P5, P10 and P20 (Kurima et al., 2002). An internal control standard, Gapdh, was measured in the same sample. Reprinted with permission from *Nature Genetics*.

and vestibule (among other places) (Bermingham et al., 1999). A very thorough review of mouse knockouts with inner ear defects was recently published (Anagnostopoulos, 2002).

Inner Ear Development and Hair Cell Organization

One of the mouse's greatest contributions to the inner ear research is the ability to study the expression of a gene or protein during embryonic development and hair cell differentiation, as well as morphology. This can be done in one or more ways. The first is by detection of messenger RNA (mRNA) derived from the inner ear by extracting inner ear or cochlear RNA and performing reverse-transcription (RT)-polymerase chain reaction (PCR), using primers specific for one's gene of interest (Fig. 2). Real-time PCR provides a quantitative (or semi-quantitative) method for evaluating the level of mRNA in specific tissues (Walker, 2002). Levels of *Myo15a*, *Tmc1*, *Tmc2*, and *Pres* were shown to be very low between embryonic day (E) 14 and postnatal day (P) 0 and peak at P10 (Kurima et al., 2002). *Myo15a* and *Tmc1* mutations are associated with deafness in *sh2*, and *dn* and *Bth* mice, respectively, as well as in humans (Table 1). A second method is in situ hybridization, where a radioactive or non-radioactive (colorimetric) label is incorporated into the DNA derived from a portion of the gene of interest, and hybridized to a whole embryo or cochlea or tissue section (Fig. 3). A third method is immunofluorescence, where an antibody is labeled to a dissected cochlea or tissue section (Fig. 4). A study of the

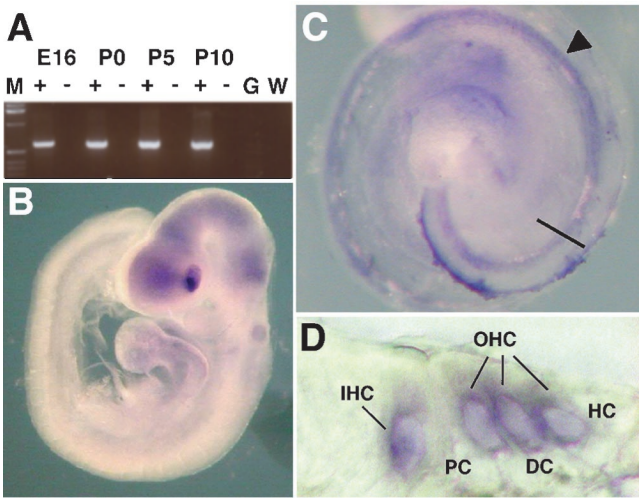

Figure 3. Detection of mRNA expression. (A) RT-PCR of *Ush3a* mRNA in temporal bones at various stages. Amplifications were carried out with (+) and without RT on cochlear RNA, on mouse genomic DNA (G), and a water control (W). Demonstration of whole mount in situ hybridization with the *Ush3a* antisense probe in a (B) mouse embryo at E9 and in a (C) cochlea at E16. Detection was performed with a digoxigenin-labeled *Ush3a* RNA probe. (D) Sectioning through the cochleae revealed specific hybridization in the inner (IHC) and outer hair cells (OHC) of the organ of Corti, but no hybridization was detected in the supporting cells of the organ of Corti, including the Deiters' cells (DC), the pillar cells (PC), and the Hensen cells (HC). The only other hybridization found in the cochlea was in the spiral ganglion cells containing the primary neurons that innervate the cochlear sensory epithelia (not shown). Mutations in the *USH3A* gene are associated with Ushers syndrome type 3 (Adato et al., 2002; Joensuu et al., 2001). Scale bar, 10 μm. Reprinted with permission from the *European Journal of Human Genetics*.

localization of myosin VIIa, harmonin b, and cadherin 23 in differentiating hair cells, along with cell culture-transfection studies, demonstrated that the three proteins work together to form the

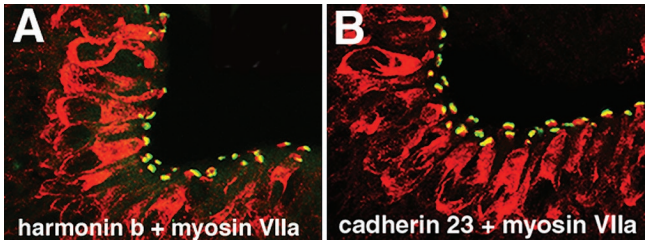

Figure 4. Detection of protein expression with antibodies in the inner ear. The example shown is that of mouse vestibular hair cells at E14. At this stage, (A) harmonin b (green) is localized to the apical surface of hair cells and myosin VIIa (red) labels the entire hair cell. (B) Cadherin 23 is also localized at the apical surface of the hair cells (green). Yellow demonstrates expression of both proteins (Boeda et al., 2002). Reprinted with permission from the *EMBO Journal*.

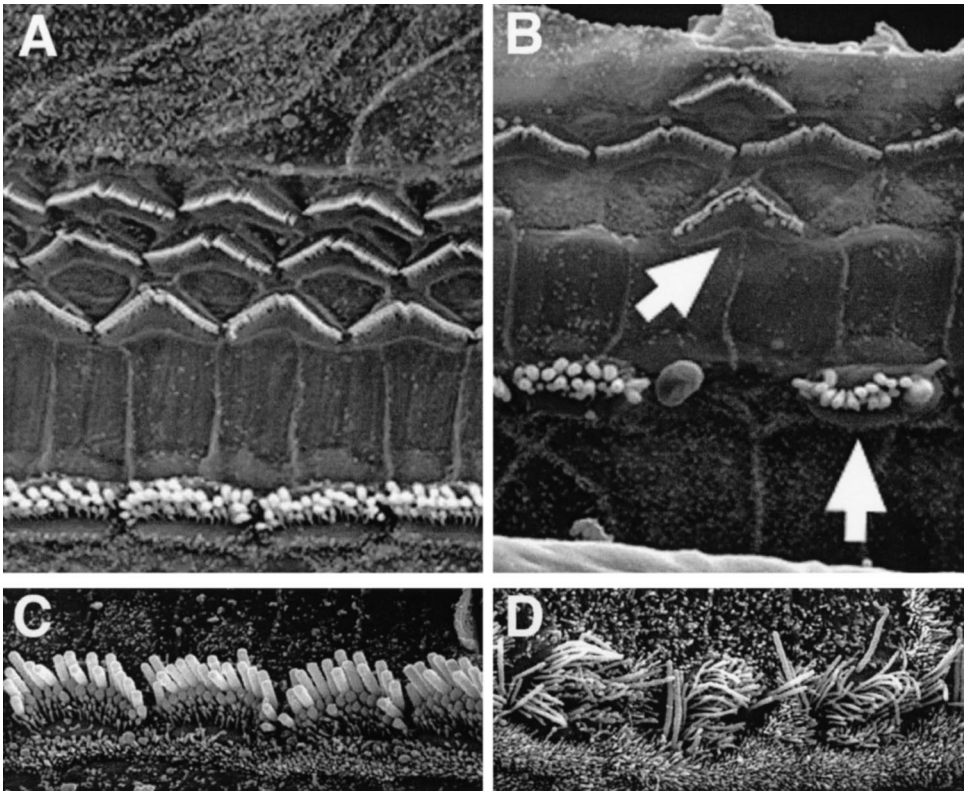

Figure 5. Scanning electron micrographs (SEM) of mouse inner ears. (A) SEM of wild type and (B) ENU-induced Beethoven (*Bth/+*) organ of Corti at P30, demonstrating hair cell degeneration (arrows) in the *Bth/+* cochlea (Vreugde et al., 2002). Reprinted with permission from *Nature Genetics*. (C) SEM of wild type and (D) Tasmanian devil (*tde*) organ of Corti at E14 shows thin stereocilia (Erven et al., 2002). Reprinted with permission from the *European Journal of Neuroscience*.

hair cell bundle (Boeda et al., 2002). Each is associated with syndromic and/or nonsyndromic hearing loss; mutations in myosin VIIA are responsible for Usher syndrome type IB (USH1B) and DFNB2/DFNA11, mutations in harmonin for USH1C, and mutations in cadherin 23 for USH1D (Friedman et al., 2003, in this issue).

Visualization of the morphology of hair cells can also provide valuable information about hair cell structure and loss (Holme & Steel, 2001). To visualize morphology in inner ears of mouse mutants, scanning electron microscopy (SEM) reveals defects in differentiation and formation of hair bundles and/or cellular architecture, and/or postnatal degeneration. A study of *Bth* mice, for example, revealed progressive hair cell degeneration, suggesting this mouse to be an appropriate model for this type of hearing loss in humans; the discovery of a mutation in *Tmc1*, the mouse orthologue of the DFNA36/*TMC1* gene, confirmed this hypothesis (Fig. 5A, B) (Vreugde et al., 2002). Abnormally thin stereocilia are shown by scanning electron microscopy (SEM) in Tasmanian devil (*tde*) mutants (see below; Fig. 5C, D).

What We Have Learned from the Mouse

Mouse mutants not only provide us with identification of genes, but even more important, they supply us with a detailed account of protein interaction and insight into developmental and functional pathways.

Norrie disease, an X-linked recessive syndromic form of deafness, is also characterized by blindness and mental retardation. In the inner ear, NDP, the gene responsible for Norrie disease, is expressed in spiral ganglion and stria vascularis. The null mutation of NDP leads to stria vascularis pathology and a reduction in vessel size, suggesting that the role of norrin is to link the cochlea with its vasculature (Rehm et al., 2002).

Connexin 26 mutations lead to the most prevalent form of non-syndromic hearing loss, but details regarding the pathology of connexin 26 malfunction in hair cells has remained elusive until recently. The connexin 26 knock-out mice die during embryogenesis due to differences in placental layers between humans and mice (Gabriel et al., 1998). Connexin 26 was inactivated using the Cre-*lox P* recombination system (Kuhn et al., 1995) in order to target the epithelial gap junction network that contains supporting cells and flanking epithelial cells. Removal of connexin 26 in the epithelial network where this protein is expressed led to cell death of supporting cells of the inner hair cells (Cohen-Salmon et al., 2002). Therefore the connexin 26 epithelial gap junction network in the cochlea is essential for hearing. Prevention of apoptosis in connexin 26 deafness may form the basis for a therapeutic approach.

More Genes Required

Considering that there are more than 90 deafness loci mapped in humans and only 30 of these genes have been cloned (updated regularly at the Hereditary Hearing Loss Homepage; http://dnalab-www.uia.ac.be/dnalab/hhh/index.html), there is clearly a need for the discovery of new genes in the auditory and vestibular pathway. Most compelling, a thorough morphological characterization of the mouse phenotype can be done before a gene is cloned, so that once the gene is found, essential information about the gene's product function is already known. An ever-growing number of reports describe the morphological phenotype of the inner ears of mouse mutants, but the responsible gene remains elusive. Nevertheless, the descriptions have provided important insights regarding hair cell development and stereociliar formation. The Tasmanian devil (*tde*) mutation was caused by insertional mutagenesis of a transgene and disruption of an as-yet unidentified gene. Homozygous mice for the insertion exhibit circling and hearing loss (Erven et al., 2002). Hair cell stereocilia are much thinner than normal and the hair cells become disorganized and eventually die (Fig. 5C, D). Therefore the *tde* gene may disrupt proper formation of actin filaments, either directly or through interaction with other actin-associated proteins. The hair cells of whirler (*wi*), a recessive mouse mutant whose phenotype is deaf and circling, have been analyzed extensively. Stereocilia are half the size of their normal counterparts, which appears to manifest itself by a reduction in their elongation after P1 (Holme, Kiernan, Brown, & Steel, 2002). Since *Myo15a* is expressed in stereocilia during elongation (Anderson et al., 2000), the *wi* and *sh2* gene products may interact.

In addition, several mouse knock-outs have been made that demonstrate that the targeted gene has a vital role in the inner ear, although mutations in these genes have not been identified in humans. Nevertheless, these mice identify key roles for these genes in the development of inner ear structures. A null mutation in *Nkx5–1*, a homeobox gene expressed in vestibular structures throughout development, leads to circling and hyperactive activity (Hadrys, Braun, Rinkwitz-Brandt, Arnold, & Bober, 1998). As there is a severe loss or reduction in the formation of the semicircular canals in these mice, the circling appears due to defects in the sensory organ and not to abnormalities in the central nervous system (although these cannot be completely ruled out). Gene-targeted mutagenesis of the *Barhl1* homeobox gene leads to severe to profound hearing loss with no associated vestibular dysfunction (Li, Price, Cahill, Ryugo, Shen, & Xiang, 2002). Progressive cochlear hair cell degeneration is observed, leading to the conclusion that *Barhl1* is essential for long-term maintenance of cochlear hair cells. Neurotrophins have been implicated in inner ear development and the knock-outs of BDNF and NT-3 provide compelling evidence from this perspective (Ernfors, Van De Water, Loring, & Jaenisch, 1995). Sensory organs from the cochlea and vestibular inner ear are innervated by afferents from spiral ganglion neurons and vestibular ganglion neurons, respectively. Trophic factors, including the neurotrophins, support the survival of these neurons (reviewed in Fritzsch et al., 2002). A null mutation in BDNF leads to loss of vestibular ganglion neurons and afferents, whereas a null mutation in NT-3 leads to loss of mostly spiral ganglion neurons and afferents. Gene-targeted mutagenesis of the *Slc12a7* gene encoding the K+/Cl− co-transporter Kcc4 leads to deafness and renal tubular acidosis (Boettger, Hubner, Maier, Rust, Beck, & Jentsch, 2002). This discovery has led to the hypothesis that Kcc4 is crucial for K+ recycling by transporting K+ from OHC to supporting Deiters' cells. The BETA2/NeuroD1 gene, a basic helix-loop-helix transcription factor up-regulates insulin gene expression and is essential for the development of the brain and pancreas. Surprisingly, a null mutation in the mice leads to circling and deafness and further investigation revealed that this phenotype is caused by a decline of sensory neurons in the cochlear-vestibular ganglion (CVG), as well as abnormalities in differentiation and patterning of the sensory epithelium (Liu et al., 2000). Yet another null mutation in a transcription factor, *FoxI1*, leads to an absence of cochlear and vestibular structures, which are replaced with a cavity (Hulander, Wurst, Carlsson, & Enerback, 1998). Most interesting, each of the above knock-outs demonstrates a crucial role of a different pathway and/or cell type in the inner ear, demonstrating how together, these mice provide a comprehensive picture of inner ear development and function.

CONCLUSION

Hundreds of mouse models are now known to have hearing and/or balance defects, and each one 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. Regardless, it is clear that using this mammal to study the human inner ear has and will continue to make a tremendous impact on the field.

ACKNOWLEDGMENTS:

Many thanks to the following for sharing figures for this review: Alexandra Erven, Karen Steel, Christine Petit, Dominique Weil, Aziz El-Amraoui, Sarah Vreugde, Andrew Griffith and to Ronna Hertzano and Nadav Ahituv for helpful comments. Research on mouse models for deafness in the Avraham laboratory is supported by the National Organization for Hearing Research Foundation (NOHR), the Israel Ministry of Health, and the Israel Science Foundation.

Address for correspondence: Karen B. Avraham, Ph.D., Department of Human Genetics and Molecular Medicine, Sackler School of Medicine, Tel Aviv University, Tel Aviv 69978 Israel. E-mail: karena@post.tau.ac.il.

Received January 21, 2003; accepted April 7, 2003

REFERENCES

Adato, A., Vreugde, S., Joensuu, T., Avidan, N., Hamalainen, R., Belenkiy, O., Olender, T., Bonne-Tamir, B., Ben-Asher, E., Espinos, C., Millan, J. M., Lehesjoki, A. E., Flannery, J. G., Avraham, K. B., Pietrokovski, S., Sankila, E. M., Beckmann, J. S., & Lancet, D. (2002). USH3A transcripts encode clarin-1, a four-transmembrane-domain protein with a possible role in sensory synapses. *European Journal of Human Genetics*, *10*, 339–350.

Ahmed, Z. M., Morell, R. J., Riazuddin, S., Gropman, A., Shaukat, S., Ahmad, M. M., Mohiddin, S. A., Fananapazir, L., Caruso, R. C., Husnain, T., Khan, S. N., Griffith, A. J., Friedman, T. B., & Wilcox, E. R. (2003). Mutations of *MYO6* are associated with recessive deafness, DFNB37. *American Journal of Human Genetics*, *72*, 1315–1322.

Alagramam, K. N., Murcia, C. L., Kwon, H. Y., Pawlowski, K. S., Wright, C. G., & Woychik, R. P. (2001). The mouse Ames waltzer hearing-loss mutant is caused by mutation of *Pcdh15*, a novel protocadherin gene. *Nature Genetics*, *27*, 99–102.

Anagnostopoulos, A. V. (2002). A compendium of mouse knockouts with inner ear defects. *Trends in Genetics*, *18*, 499.

Avraham, K. B., Hasson, T., Steel, K. P., Kingsley, D., M., Russell, L. B., Mooseker, M. S., Copeland, N. G., & Jenkins, N. A. (1995). The mouse Snell's waltzer deafness gene encodes an unconventional myosin required for structural integrity of inner ear hair cells. *Nature Genetics*, *11*, 369–375.

Berger, W., van de Pol, D., Bachner, D., Oerlemans, F., Winkens, H., Hameister, H., Wieringa, B., Hendriks, W., & Ropers, H. H. (1996). An animal model for Norrie disease (ND): Gene targeting of the mouse ND gene. *Human Molecular Genetics*, *5*, 51–59.

Bermingham, N. A., Hassan, B. A., Price, S. D., Vollrath, M. A., Ben-Arie, N., Eatock, R. A., Bellen, H. J., Lysakowski, A., & Zoghbi, H. Y. (1999). Math1: An essential gene for the generation of inner ear hair cells. *Science*, *284*, 1837–1841.

Boeda, B., El-Amraoui, A., Bahloul, A., Goodyear, R., Daviet, L., Blanchard, S., Perfettini, I., Fath, K. R., Shorte, S., Reiners, J., Houdusse, A., Legrain, P., Wolfrum, U., Richardson, G., & Petit, C. (2002). Myosin VIIa, harmonin and cadherin 23, three Usher I gene products that cooperate to shape the sensory hair cell bundle. *EMBO Journal*, *21*, 6689–6699.

Boeda, B., Weil, D., & Petit, C. (2001). A specific promoter of the sensory cells of the inner ear defined by transgenesis. *Human Molecular Genetics*, *10*, 1581–1589.

Boettger, T., Hubner, C. A., Maier, H., Rust, M. B., Beck, F. X., & Jentsch, T. J. (2002). Deafness and renal tubular acidosis in mice lacking the K-Cl co-transporter Kcc4. *Nature*, *416*, 874–878.

Bromham, L., Phillips, M. J., & Penny, D. (1999). Growing up with dinosaurs: molecular dates and the mammalian radiation. *Trends in Ecology and Evolution*, *14*, 113–118.

Cohen-Salmon, M., Ott, T., Michel, V., Hardelin, J. P., Perfettini, I., Eybalin, M., Wu, T., Marcus, D. C., Wangemann, P., Willecke, K., & Petit, C. (2002). Targeted ablation of connexin26 in the inner ear epithelial gap junction network causes hearing impairment and cell death. *Current Biology*, *12*, 1106–1111.

Copeland, N. G., Jenkins, N. A., & O'Brien, S. J. (2002). Genomics. Mmu 16—comparative genomic highlights. *Science*, *296*, 1617–1618.

Cosgrove, D., Meehan, D. T., Grunkemeyer, J. A., Kornak, J. M., Sayers, R., Hunter, W. J., & Samuelson, G. C. (1996). Collagen COL4A3 knockout: a mouse model for autosomal Alport syndrome. *Genes and Development*, *10*, 2981–2992.

Deol, M. S. (1980). Genetic malformations of the inner ear in the mouse and in man. *Birth Defects Original Article Series*, *16*, 243–261.

Deol, M. S., & Green, M. C. (1966). Snell's waltzer, a new mutation affecting behaviour and the inner ear in the mouse. *Genetic Research*, *8*, 339–345.

Di Palma, F., Holme, R. H., Bryda, E. C., Belyantseva, I. A., Pellegrino, R., Kachar, B., Steel, K. P., & Noben-Trauth, K. (2001). Mutations in *Cdh23*, encoding a new type of cadherin, cause stereocilia disorganization in waltzer, the mouse model for Usher syndrome type 1D. *Nature Genetics*, *27*, 103–107.

Dobrovolskaia-Zavasdkaia, N. (1928). L'irradiation des testicules et l'heredite chez la souris. *Archives Biology*, *38*, 457–501.

Epstein, D. J., Vekemans, M., & Gros, P. (1991). Splotch (*Sp2H*), a mutation affecting development of the mouse neural tube, shows a deletion within the paired homeodomain of Pax-3. *Cell*, *67*, 767–774.

Erkman, L., McEvilly, R. J., Luo, L., Ryan, A. K., Hooshmand, F., O'Connell, S. M., Keithley, E. M., Rapaport, D. H., Ryan, A. F., & Rosenfeld, M. G. (1996). Role of transcription factors Brn-3.1 and Brn-3.2 in auditory and visual system development. *Nature*, *381*, 603–606.

Ernfors, P., Van De Water, T., Loring, J., & Jaenisch, R. (1995). Complementary roles of BDNF and NT-3 in vestibular and auditory development. *Neuron*, *14*, 1153–1164.

Erven, A., Skynner, M. J., Okumura, K., Takebayashi, S., Brown, S. D., Steel, K. P., & Allen, N. D. (2002). A novel stereocilia defect in sensory hair cells of the deaf mouse mutant Tasmanian devil. *European Journal of Neuroscience*, *16*, 1433–1441.

Everett, L. A., Belyantseva, I. A., Noben-Trauth, K., Cantos, R., Chen, A., Thakkar, S. I., Hoogstraten-Miller, S. L., Kachar, B., Wu, D. K., & Green, E. D. (2001). Targeted disruption of mouse *Pds* provides insight about the inner-ear defects encountered in Pendred syndrome. *Human Molecular Genetics*, *10*, 153–161.

Friedman, T. B., Schultz, J. M., Ben-Yosef, T., Pryor, S. P., Lagziel, A., Fisher, R. A., Wilcox, E. R., Riazuddin, S., Ahmed, Z. M., Belyantseva, I. A., & Griffith, A. J. (2003). Recent advances in the understanding of syndromic forms of hearing loss. *Ear and Hearing*, *24*.

Fritzsch, B., Beisel, K. W., Jones, K., Farinas, I., Maklad, A., Lee, J., & Reichardt, L. F. (2002). Development and evolution of inner ear sensory epithelia and their innervation. *Journal of Neurobiology*, *53*, 143–156.

Gardner, J. M., Nakatsu, Y., Gondo, Y., Lee, S., Lyon, M. F., King, R. A., & Brilliant, M. H. (1992). The mouse pink-eyed dilution gene: association with human Prader-Willi and Angelman syndromes. *Science*, *257*, 1121–1124.

Gates, W. H. (1929). Linkage of the characters albinism and shaker in the house mouse. *Anatomical Record*, *41*, 104.

Gibson, F., Walsh, J., Mburu, P., Varela, A., Brown, K. A., Antonio, M., Beisel, K. W., Steel, K. P., & Brown, S. D. (1995).

A type VII myosin encoded by the mouse deafness gene shaker-1. *Nature*, *374*, 62–64.

Gordon, J. W., Scangos, G. A., Plotkin, D. J., Barbosa, J. A., & Ruddle, F. H. (1980). Genetic transformation of mouse embryos by microinjection of purified DNA. *Proceedings of the National Academy of Sciences USA*, *77*, 7380–7384.

Hadrys, T., Braun, T., Rinkwitz-Brandt, S., Arnold, H. H., & Bober, E. (1998). Nkx5–1 controls semicircular canal formation in the mouse inner ear. *Development*, *125*, 33–39.

Hardisty, R. E., Erven, A., Logan, K., Morse, S., Guionaud, S., Sancho-Oliver, S., Jackie Hunter, A., Brown, S., D., & Steel, K. P. (2002). The deaf mouse mutant Jeff (*Jf*) is a single gene model of otitis media. *Journal of the Association for Research in Otolaryngology*, DOI: 10.1007/s10162-002-3015-9.

Hodgkinson, C. A., Moore, K. J., Nakayama, A., Steingrimsson, E., Copeland, N. G., Jenkins, N. A., & Arnheiter, H. (1993). Mutations at the mouse microphthalmia locus are associated with defects in a gene encoding a novel basic-helix-loop-helix-zipper protein. *Cell*, *74*, 395–404.

Holme, R. H., Kiernan, B. W., Brown, S. D., & Steel, K. P. (2002). Elongation of hair cell stereocilia is defective in the mouse mutant whirler. *Journal of Comparative Neurology*, *450*, 94–102.

Holme, R. H., & Steel, K. P. (2001). Hair cell function—it's all a matter of organization. *Trends in Molecular Medicine*, *7*, 138.

Hulander, M., Wurst, W., Carlsson, P., & Enerback, S. (1998). The winged helix transcription factor Fkh10 is required for normal development of the inner ear. *Nature Genetics*, *20*, 374–376.

Joensuu, T., Hamalainen, R., Yuan, B., Johnson, C., Tegelberg, S., Gasparini, P., Zelante, L., Pirvola, U., Pakarinen, L., Lehesjoki, A. E., de la Chapelle, A., & Sankila, E. M. (2001). Mutations in a novel gene with transmembrane domains underlie Usher syndrome type 3. *American Journal of Human Genetics*, *69*, 673–684.

Johnson, K. R., Cook, S. A., Erway, L. C., Matthews, A. N., Sanford, L. P., Paradies, N. E., & Friedman, R. A. (1999). Inner ear and kidney anomalies caused by IAP insertion in an intron of the *Eya1* gene in a mouse model of BOR syndrome. *Human Molecular Genetics*, *8*, 645–653.

Joyner, A. L. (2000). *Gene Targeting: A Practical Approach*. Oxford, Oxford University Press.

Justice, M. J., Noveroske, J. K., Weber, J. S., Zheng, B., & Bradley, A. (1999). Mouse ENU mutagenesis. *Human Molecular Genetics*, *8*, 1955–1963.

Kiernan, A. E., Ahituv, N., Fuchs, H., Balling, R., Avraham, K. B., Steel, K. P., & Hrabe de Angelis, M. (2001). The Notch ligand Jagged1 is required for inner ear sensory development. *Proceedings of the National Academy of Sciences USA*, *98*, 3873–3878.

Kuhn, R., Schwenk, F., Aguet, M., & Rajewsky, K. (1995). Inducible gene targeting in mice. *Science*, *269*, 1427–1429.

Kurima, K., Peters, L. M., Yang, Y., Riazuddin, S., Ahmed, Z. M., Naz, S., Arnaud, D., Drury, S., Mo, J., Makishima, T., Ghosh, M., Menon, P. S., Deshmukh, D., Oddoux, C., Ostrer, H., Khan, S., Riazuddin, S., Deininger, P. L., Hampton, L. L., Sullivan, S. L., Battey, J. F., Jr., Keats, B. J., Wilcox, E. R., Friedman, T. B., & Griffith, A. J. (2002). Dominant and recessive deafness caused by mutations of a novel gene, *TMC1*, required for cochlear hair-cell function. *Nature Genetics*, *30*, 277–284.

Kwon, B. S., Haq, A. K., Pomerantz, S. H., & Halaban, R. (1987). Isolation and sequence of a cDNA clone for human tyrosinase that maps at the mouse c-albino locus. *Proceedings of the National Academy of Sciences USA*, *84*, 7473–7477.

Lander, E. S., et al. (2001). Initial sequencing and analysis of the human genome. *Nature*, *409*, 860–921.

Lee, M. P., Ravenel, J. D., Hu, R. J., Lustig, L. R., Tomaselli, G., Berger, R. D., Brandenburg, S. A., Litzi, T. J., Bunton, T. E., Limb, C., Francis, H., Gorelikow, M., Gu, H., Washington, K., Argani, P., Goldenring, J. R., Coffey, R., J., & Feinberg, A. P. (2000). Targeted disruption of the *Kvlqt1* gene causes deafness and gastric hyperplasia in mice. *Journal of Clinical Investigation*, *106*, 1447–1455.

Li, S., Price, S. M., Cahill, H., Ryugo, D. K., Shen, M. M., & Xiang, M. (2002). Hearing loss caused by progressive degeneration of cochlear hair cells in mice deficient for the *Barhl1* homeobox gene. *Development*, *129*, 3523–3532.

Liu, M., Pereira, F. A., Price, S. D., Chu, M. J., Shope, C., Himes, D., Eatock, R. A., Brownell, W. E., Lysakowski, A., & Tsai, M. J. (2000). Essential role of BETA2/NeuroD1 in development of the vestibular and auditory systems. *Genes and Development*, *14*, 2839–2854.

McGuirt, W. T., Prasad, S. D., Griffith, A. J., Kunst, H. P., Green, G. E., Shpargel, K. B., Runge, C., Huybrechts, C., Mueller, R. F., Lynch, E., King, M.-C., Brunner, H. G., Cremers, C. W., Takanosu, M., Li, S. W., Arita, M., Mayne, R., Prockop, D. J., Van Camp, G., & Smith, R. J. (1999). Mutations in COL11A2 cause non-syndromic hearing loss (DFNA13). *Nature Genetics*, *23*, 413–419.

Melchionda, S., Ahituv, N., Bisceglia, L., Sobe, T., Glaser, F., Rabionet, R., Arbones, M. L., Notarangelo, A., Di Iorio, E., Carella, M., Zelante, L., Estivill, X., Avraham, K. B., & Gasparini, P. (2001). *MYO6*, the human homologue of the gene responsible for deafness in Snell's waltzer mice, is mutated in autosomal dominant nonsyndromic hearing loss. *Am J Human Genetics*, *69*, 635–640.

Minowa, O. Ikeda, K., Sugitani, Y., Oshima, T., Nakai, S., Katori, Y., Suzuki, M., Furukawa, M., Kawase, T., Zheng, Y., Ogura, M., Asada, Y., Watanabe, K., Yamanaka, H., Gotoh, S., Nishi-Takeshima, M., Sugimoto, T., Kikuchi, T., Takasaka, T., & Noda, T. (1999). Altered cochlear fibrocytes in a mouse model of DFN3 nonsyndromic deafness. *Science*, *285*, 1408–1411.

Mitchem, K. L., Hibbard, E., Beyer, L. A., Bosom, K., Dootz, G. A., Dolan, D. F., Johnson, K. R., Raphael, Y., & Kohrman, D. C. (2002). Mutation of the novel gene Tmie results in sensory cell defects in the inner ear of spinner, a mouse model of human hearing loss DFNB6. *Human Molecular Genetics*, *11*, 1887–1898.

Nadeau, J. H., Singer, J. B., Matin, A., & Lander, E. S. (2000). Analysing complex genetic traits with chromosome substitution strains. *Nature Genetics*, *24*, 221–225.

Naz, S., Riazuddin, S., Riazuddin, S., Griffith, A. J., Friedman, T. B., & Wilcox, E. R. (2002). A mutation of ESPN causes autosomal recessive nonsyndromic hearing loss, DFNB36. *American Journal of Human Genetics*, *71S*, 511.

Perez-Losada, J., Sanchez-Martin, M., Rodriguez-Garcia, A., Sanchez, M. L., Orfao, A., Flores, T., & Sanchez-Garcia, I. (2002). Zinc-finger transcription factor Slug contributes to the function of the stem cell factor c-kit signaling pathway. *Blood*, *100*, 1274–1286.

Phippard, D., Lu, L., Lee, D., Saunders, J. C., & Crenshaw, E. B., 3rd. (1999). Targeted mutagenesis of the POU-domain gene Brn4/Pou3f4 causes developmental defects in the inner ear. *Journal of Neuroscience*, *19*, 5980–5989.

Probst, F. J., Fridell, R. A., Raphael, Y., Saunders, T. L., Wang, A., Liang, Y., Morell, R. J., Touchman, J. W., Lyons, R. H., Noben-Trauth, K., Friedman, T. B., & Camper, S. A. (1998). Correction of deafness in shaker-2 mice by an unconventional myosin in a BAC transgene. *Science*, *280*, 1444–1447.

Rehm, H. L., Zhang, D. S., Brown, M. C., Burgess, B., Halpin, C., Berger, W., Morton, C. C., Corey, D. P., & Chen, Z. Y. (2002). Vascular defects and sensorineural deafness in a mouse model of Norrie disease. *Journal of Neuroscience*, *22*, 4286–4292.

Russell, L. B. (1971). Definition of functional units in a small chromosomal segment of the mouse and its use in interpreting the nature of radiation-induced mutations. *Mutation Research*, *11*, 107–123.

Shin, M. K., Russell, L. B., & Tilghman, S. M. (1997). Molecular characterization of four induced alleles at the *Ednrb* locus. *Proceedings of the National Academy of Sciences USA*, *94*, 13105–13110.

Shizuya, H., Birren, B., Kim, U. J., Mancino, V., Slepak, T., Tachiiri, Y., & Simon, M. (1992). Cloning and stable maintenance of 300-kilobase-pair fragments of human DNA in Escherichia coli using an F-factor-based vector. *Proceedings of the National Academy of Sciences USA*, *89*, 8794–8797.

Silver, L. M. (1995). *Mouse Genetics: Concepts and Applications*. Oxford, Oxford University Press.

Snell, G. D. (1945). Linkage of jittery and waltzing in the mouse. *Journal of Heredity*, *36*, 279–280.

Steel, K. P., & Bock, G. R. (1980). The nature of inherited deafness in deafness mice. *Nature*, *288*, 159–161.

Tsai, H., Hardisty, R. E., Rhodes, C., Kiernan, A. E., Roby, P., Tymowska-Lalanne, Z., Mburu, P., Rastan, S., Hunter, A. J., Brown, S. D., & Steel, K. P. (2001). The mouse slalom mutant demonstrates a role for Jagged1 in neuroepithelial patterning in the organ of Corti. *Human Molecular Genetics*, *10*, 507–512.

Vahava, O., Morell, R., Lynch, E. D., Weiss, S., Kagan, M. E., Ahituv, N., Morrow, J. E., Lee, M., K., Skvorak, A. B., Morton, C. C., Blumenfeld, A., Frydman, M., Friedman, T. B., King, M. C., & Avraham, K. B. (1998). Mutation in transcription factor *POU4F3* associated with inherited progressive hearing loss in humans. *Science*, *279*, 1950–1954.

Van Laer, L., Cryns, K., Smith, R. J. H., & Van Camp, G. (2003). Nonsyndromic hearing loss. *Ear and Hearing*, *24*.

Vetter, D. E., Mann, J. R., Wangemann, P., Liu, J., McLaughlin, K. J., Lesage, F., Marcus, D. C., Lazdunski, M., Heinemann, S. F., & Barhanin, J. (1996). Inner ear defects induced by null mutation of the *isk* gene. *Neuron*, *17*, 1251–1264.

Vreugde, S., Erven, A., Kros, C. J., Marcotti, W., Fuchs, H., Kurima, K., Wilcox, E. R., Friedman, T. B., Griffith, A. J., Balling, R., Hrabe De Angelis, M., Avraham, K. B., & Steel, K. P. (2002). Beethoven, a mouse model for dominant, progressive hearing loss DFNA36. *Nature Genetics*, *30*, 257–258.

Walker, N. J. (2002). Tech.Sight. A technique whose time has come. *Science*, *296*, 557–559.

Wang, A., Liang, Y., Fridell, R. A., Probst, F. J., Wilcox, E. R., Touchman, J. W., Morton, C. C., Morell, R. J., Noben-Trauth, K., Camper, S. A., & Friedman, T. B. (1998). Association of unconventional myosin *MYO15* mutations with human nonsyndromic deafness DFNB3. *Science*, *280*, 1447–1451.

Waterston, R. H., et al. (2002). Initial sequencing and comparative analysis of the mouse genome. *Nature*, *420*, 520–562.

Wilson, S. M., Householder, D. B., Coppola, V., Tessarollo, L., Fritzsch, B., Lee, E. C., Goss, D., Carlson, G. A., Copeland, N. G., & Jenkins, N. A. (2001). Mutations in *Cdh23* cause nonsyndromic hearing loss in waltzer mice. *Genomics*, *74*, 228–233.

Xiang, M., Gan, L., Li, D., Chen, Z. Y., Zhou, L., O'Malley, B. W., Jr., Klein, W., & Nathans, J. (1997). Essential role of POU-domain factor Brn-3c in auditory and vestibular hair cell development. *Proceedings of the National Academy of Sciences USA*, *94*, 9445–9450.

Xu, P. X., Adams, J., Peters, H., Brown, M. C., Heaney, S., & Maas, R. (1999). Eya1-deficient mice lack ears and kidneys and show abnormal apoptosis of organ primordia. *Nature Genetics*, *23*, 113–117.

Yang, X. W., Model, P., & Heintz, N. (1997). Homologous recombination based modification in Escherichia coli and germline transmission in transgenic mice of a bacterial artificial chromosome. *Nature Biotechnology*, *15*, 859–865.

Yasunaga, S., Grati, M., Cohen-Salmon, M., El-Amraoui, A., Mustapha, M., Salem, N., El-Zir, E., Loiselet, J., & Petit, C. (1999). A mutation in OTOF, encoding otoferlin, a FER-1-like protein, causes DFNB9, a nonsyndromic form of deafness. *Nature Genetics*, *21*, 363–369.