PNAS COMMENTARY

Emerging complexities of the mouse as a model for human hearing loss

Ryan J. Carlson[a,b] and Karen B. Avraham[c,1]

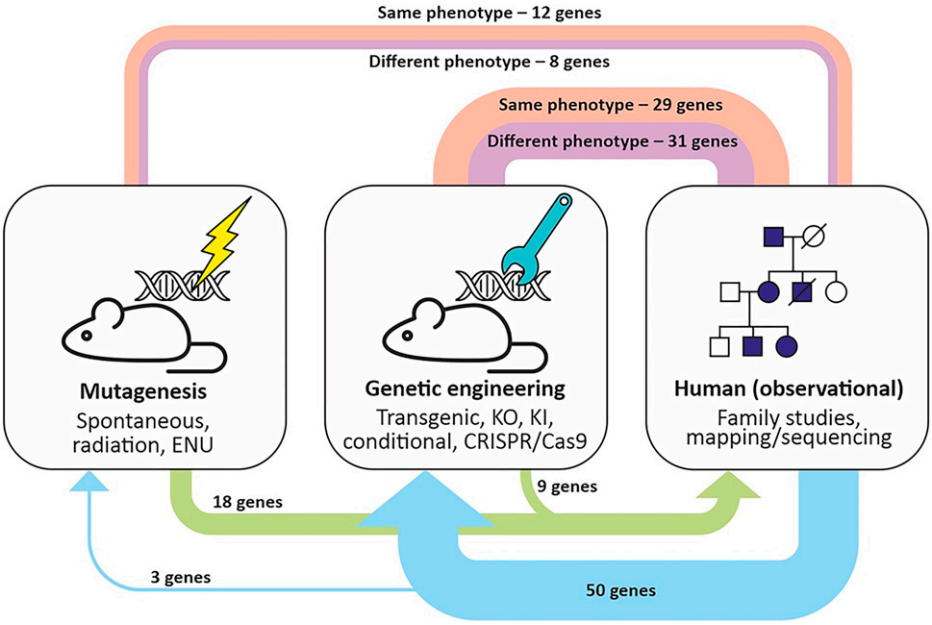

Fig. 1. Correspondence of hearing loss genotypes and phenotypes in humans and mice. (*Middle*) Approaches to gene discovery and characterization include mutagenesis in mice, genetic engineering of mice, and observational genetics studies of human families. (*Top*) Mouse models have been reported for 80 genes implicated in human nonsyndromic hearing loss. Human and mouse phenotypes were considered different if syndromic effects were present in one but not both species; if hearing losses differed substantially between species in age at onset, severity, progression, or pattern of affected sound frequencies; or if mouse mutations comparable to human alleles led to preweaning lethality in mice. (*Bottom*) Chronology of discovery. For 27 genes (primarily those discovered early), genes for hearing loss in mouse were discovered first, and human families were subsequently discovered with hearing loss due to mutations in the same gene. For 53 genes (primarily those discovered more recently), candidate genes emerged from studies of human families and were confirmed by studies of engineered mice, or in a few cases of mice identified by mutagenesis screens. Thickness of arcs is proportional to the number of genes in each category.

In their recent publication "Mutations in *MINAR2* encoding membrane integral NOTCH2-associated receptor 2 cause deafness in humans and mice," Bademci et al. (1) studied both humans and mice to discover and characterize a gene responsible for human hearing loss. In human families they identified multiple variants in *MINAR2* that led to loss of function of the gene and were perfectly coinherited with autosomal recessive hearing loss. A mouse with loss of function of *Minar2* was known to have hearing loss (2), strongly supporting the interpretation that loss of function of the gene was also responsible for hearing loss in the human families. Further characterization of the *Minar2* mutant mice helped to elucidate the basis of this hearing loss in both species. This discovery also illustrates the complexity of the mouse as a model for human hearing loss: Mice and humans with mutations causing loss of function of *MINAR2* did not display the same overall phenotypes. Specifically, in addition to hearing loss, *Minar2* mutant mice developed motor deficits, including bradykinesia and rigidity reminiscent of Parkinson's disease (3). These motor deficits were not present in even the oldest humans with comparably severe mutations in *MINAR2*.

Since the earliest research in genetics of hearing loss, gene discovery efforts have very fruitfully integrated studies of humans and mice. In this commentary, we review this experience and suggest that the many mice now created by modern tools of gene engineering may reveal additional complex genotype–phenotype relationships between the species that will inform the biology of both.

In 1929, Lord and Gates described a mutant mouse with a peculiar behavioral phenotype including head tossing and circling behavior along with hearing loss (4). They

Author affiliations: [a]Department of Genome Sciences, University of Washington, Seattle, WA 98195; [b]Department of Medicine, University of Washington, Seattle, WA 98195; and [c]Department of Human Molecular Genetics and Biochemistry, Faculty of Medicine and Sagol School of Neuroscience, Tel Aviv University, Tel Aviv 6997801, Israel

Author contributions: R.J.C. and K.B.A. wrote the paper.

The authors declare no competing interest.

See companion article, "Mutations in *MINAR2* encoding membrane integral NOTCH2-associated receptor 2 cause deafness in humans and mice," 10.1073/pnas.2204084119.

[1]To whom correspondence may be addressed. Email: karena@tauex.tau.ac.il.

Published August 12, 2022.

noted that the phenotype followed an autosomal recessive inheritance pattern, (correctly) hypothesized that the features were due to a homozygous mutation, and named the mutant *shaker-1*. In the years since, inbred mouse strains have provided the ideal genetic background for understanding recessive hearing loss phenotypes. Deaf mouse mutants were discovered by spontaneous mutation or through mutagenesis screens using radiation or, later, *N*-ethyl-*N*-nitrosourea (ENU) (5–7). These phenotypes were extensively characterized in the mouse, but this species became the dominant model for studies of mammalian hearing loss only in the 1990s, when parallel genetic mapping of human and mouse deafness loci converged on common genes. This convergence provided evidence of shared inner ear biology in the two species (8). In many cases, the mutations causing deafness in long-maintained mouse strains had occurred in genes newly implicated in human hearing loss: *shaker-1* due to mutation in *MYO7A* (4, 9), *shaker-2* due to mutation in *MYO15A* (10), and *Beethoven* due to mutation in *TMC1* (11), among others.

Bademci et al. studied both humans and mice to discover and characterize a gene responsible for human hearing loss.

The contribution of mouse models to the study of hearing loss is immense. The mouse is an ideal model for human hearing loss both because it has a short generation time (19- to 21-d gestation and sexual maturity by age 6 wk) and because the mouse cochlea is strikingly similar to that of humans and can be readily visualized via dissection and imaging (12). Mice are born without their cochlea fully developed, so their hearing is not functionally mature until ∼3 wk of age, enabling direct study and precise timing of the consequences of many mutations. Hearing in mice then ages quickly during their lifespan and can be calibrated with human aging (13, 14).

Technological advances in this century have further expanded the uses of mouse models in mammalian hearing loss research. In 2002, the mouse genome was published and the identities and differences of mouse and human genes became apparent (15). Development of conditional knockout techniques enabled creation of mouse models that were previously unattainable. For example, for *GJB2*, the gene most frequently mutated in human hearing loss, complete knockout in mice is embryonic lethal due to the critical role of *Gjb2* in transplacental nutrition of the mouse embryo (16). *GJB2* is not critical for this function in humans. Conditional knockout of *Gjb2* in the mouse inner ear was viable and mutant mice demonstrated hearing loss reminiscent of the human phenotype (17). Most recently, CRISPR-Cas9 approaches have been used to create knock-in mouse strains that carry specific mutations identified in informative human families (18). These technologies have flipped the study design of gene discovery for hearing loss. Originally, genes for hearing loss were discovered first in mice and subsequently found to be mirrored by mutations in the same genes in human families. Now, candidate mutations discovered first in human families can be engineered directly into mouse models and tested for their effects on hearing. Moreover, CRISPR gene editing is also being used for gene therapy (19).

This synergy revealed similarities and differences between the species in hearing and in pleiotropic effects of mutant genes on other organ systems. Fig. 1 illustrates some of these patterns. Nearly all genes responsible for nonsyndromic sensorineural hearing loss in humans have been studied also in a mouse model, and for most hearing loss in mouse and human are virtually the same in onset, severity, pattern, and progression. In contrast, the story is far more complex when considering syndromic features (or pleiotropic effects). For eight genes, including *MINAR2*, both species have hearing loss, but the mutant mouse demonstrates additional syndromic features not found in humans. Across different genes, syndromic features due to these genes affect a very wide range of organ systems (20). For eight other genes, both species have hearing loss, but humans display additional syndromic features not seen in mice.

This latter group contains several genes with mutations that cause Usher syndrome, which includes deafness, blindness, and balance (vestibular) problems, with wide variation in severity depending on the responsible gene. Because vestibular problems in mice are easily observable (e.g., circling behavior and head tossing), mouse mutants corresponding to human Usher syndrome genes were readily identified; for example, *shaker-1*, *waltzer*, and *whirler* are caused by mutations in *MYO7A*, *CDH23*, and *WHRN*, respectively. Interestingly, none of these mouse models had retinal degeneration, and biological changes in the eye were not nearly as severe as in humans (21). In contrast, mouse mutants corresponding to human Usher syndrome due to mutations in *USH2A* or *CLRN* do exhibit retinal degeneration (21). However, the conditions required are complex: For mice with mutations in *Clrn*, severity of vision problems depends both on the specific *Clrn* mutation (as for humans) and on the mouse's genetic background (22).

Mutations in nine other genes lead to hearing loss in both human and mouse, but the hearing losses differ in severity, progression, onset, or pattern. For example, mutations in human *SYNE4* cause high-frequency hearing loss that progresses slowly (23), with some individuals retaining near-normal low-frequency hearing up to age 50 y. In contrast, mice with comparable mutations in *Syne4* demonstrate moderately severe hearing loss across all frequencies by 15 d after birth and progress to severe/profound deafness in all frequencies by 60 d after birth [equivalent to approximately age 15 y in humans (13, 14)]. This difference could be due to species differences in the roles of the proteins of the cytoskeletal LINC complex in the inner ear or to species differences in response to physical forces on hair cells. For six genes, mutations are reported to lead to hearing loss in human families, but mice with comparably severe genotypes hear normally. For example, a recently made CRISPR-Cas9 mouse model for nonsyndromic *TBC1D24*-related hearing loss has normal hearing (24).

The first genes found to be responsible for human hearing loss were either mutant in many affected families, such as *GJB2* (25), or corresponded to a known mouse strain with a very similar hearing loss that

confirmed the human discovery, such as *MYO7A* (9) and *MYO15A* (10). In contrast, for many recent discoveries of genes for human hearing loss, no mouse model yet exists when a human candidate gene emerges from genomic analysis. For these discoveries, proof of causality of the human gene requires a combination of in vitro evaluation of effects of mutation on protein function and in vivo recreation of hearing loss. As illustrated by Fig. 1, directed genomic engineering in mice has been the tool of choice for these studies.

For 11 genes, no mouse model has been reported in the literature or by the International Mouse Phenotyping Consortium (IMPC) (20). For some of these genes, it is possible that mouse models were generated that recapitulate the human genotype but do not recapitulate the human hearing loss. Hesitation to publish negative results may explain some of these "missing mice."

The mouse is an ideal model for mammalian hearing loss and has been integral to the progress of a century of research. The present study of *MINAR2* illustrates how mouse models can be used to aid human genetics and improve our understanding of inner-ear biology. With increasing numbers of mice being engineered using modern tools, more examples of mouse–human phenotypic divergence will arise. We encourage publication of all well-engineered mouse models, whether convergent or divergent with human phenotypes, whose creation was motivated by discovery of genes for human hearing loss. These models may reveal critical biology of hearing loss specific to humans and/or reveal genes with very different functions in humans and mice. In both contexts, these exceptional mice have special roles to play.

ACKNOWLEDGMENTS. R.J.C. and K.B.A. research was supported by NIH Grant R01DC011835.

1. G. Bademci *et al.*, Mutations in *MINAR2* encoding membrane integral NOTCH2-associated receptor 2 cause deafness in humans and mice. *Proc. Natl. Acad. Sci. U.S.A.* **119**, e2204084119 (2022).
2. N. J. Ingham *et al.*, Mouse screen reveals multiple new genes underlying mouse and human hearing loss. *PLoS Biol.* **17**, e3000194 (2019).
3. R. X.-Y. Ho *et al.*, Loss of *MINAR2* impairs motor function and causes Parkinson's disease-like symptoms in mice. *Brain Commun.* **2**, fcaa047 (2020).
4. E. M. Lord, W. H. Gates, Shaker, a new mutation of the house mouse (*Mus musculus*). *Am. Nat.* **63**, 435–442 (1929).
5. M. J. Justice, J. K. Noveroske, J. S. Weber, B. Zheng, A. Bradley, Mouse ENU mutagenesis. *Hum. Mol. Genet.* **8**, 1955–1963 (1999).
6. M. S. Deol, Inherited diseases of the inner ear in man in the light of studies on the mouse. *J. Med. Genet.* **5**, 137–158 (1968).
7. K. P. Steel, G. R. Bock, The nature of inherited deafness in deafness mice. *Nature* **288**, 159–161 (1980).
8. K. K. Ohlemiller, S. M. Jones, K. R. Johnson, Application of mouse models to research in hearing and balance. *J. Assoc. Res. Otolaryngol.* **17**, 493–523 (2016).
9. D. Weil *et al.*, Defective myosin VIIA gene responsible for Usher syndrome type 1B. *Nature* **374**, 60–61 (1995).
10. A. Wang *et al.*, Association of unconventional myosin *MYO15* mutations with human nonsyndromic deafness DFNB3. *Science* **280**, 1447–1451 (1998).
11. S. Vreugde *et al.*, Beethoven, a mouse model for dominant, progressive hearing loss DFNA36. *Nat. Genet.* **30**, 257–258 (2002).
12. A. A. Dror, K. B. Avraham, Hearing loss: Mechanisms revealed by genetics and cell biology. *Annu. Rev. Genet.* **43**, 411–437 (2009).
13. Y. Narui *et al.*, Development of distortion product otoacoustic emissions in C57BL/6J mice. *Int. J. Audiol.* **48**, 576–581 (2009).
14. R. Gagnon, C. Hunse, L. Carmichael, F. Fellows, J. Patrick, Human fetal responses to vibratory acoustic stimulation from twenty-six weeks to term. *Am. J. Obstet. Gynecol.* **157**, 1375–1381 (1987).
15. R. H. Waterston *et al.*; Mouse Genome Sequencing Consortium, Initial sequencing and comparative analysis of the mouse genome. *Nature* **420**, 520–562 (2002).
16. H. D. Gabriel *et al.*, Transplacental uptake of glucose is decreased in embryonic lethal connexin26-deficient mice. *J. Cell Biol.* **140**, 1453–1461 (1998).
17. M. Cohen-Salmon *et al.*, Targeted ablation of connexin26 in the inner ear epithelial gap junction network causes hearing impairment and cell death. *Curr. Biol.* **12**, 1106–1111 (2002).
18. J. Wang *et al.*, A humanized mouse model, demonstrating progressive hearing loss caused by *MYO6* p.C442Y, is inherited in a semi-dominant pattern. *Hear. Res.* **379**, 79–88 (2019).
19. B. György *et al.*, Allele-specific gene editing prevents deafness in a model of dominant progressive hearing loss. *Nat. Med.* **25**, 1123–1130 (2019).
20. V. Muñoz-Fuentes *et al.*; IMPC consortium, The International Mouse Phenotyping Consortium (IMPC): A functional catalogue of the mammalian genome that informs conservation. *Conserv. Genet.* **19**, 995–1005 (2018).
21. D. S. Williams, Usher syndrome: Animal models, retinal function of Usher proteins, and prospects for gene therapy. *Vision Res.* **48**, 433–441 (2008).
22. G. Tian, R. Lee, P. Ropelewski, Y. Imanishi, Impairment of vision in a mouse model of usher syndrome type III. *Invest. Ophthalmol. Vis. Sci.* **57**, 866–875 (2016).
23. H. F. Horn *et al.*, The LINC complex is essential for hearing. *J. Clin. Invest.* **123**, 740–750 (2013).
24. R. Tona *et al.*, Mouse models of human pathogenic variants of *TBC1D24* associated with non-syndromic deafness DFNB86 and DFNA65 and syndromes involving deafness. *Genes (Basel)* **11**, 1122 (2020).
25. D. P. Kelsell *et al.*, Connexin 26 mutations in hereditary non-syndromic sensorineural deafness. *Nature* **387**, 80–83 (1997).