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Genetically modified pigs: Emerging animal models for hereditary hearing loss

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ABSTRACT

Hereditary hearing loss (HHL), a genetic disorder that impairs auditory function, significantly affects quality of life and incurs substantial economic losses for society. To investigate the underlying causes of HHL and evaluate therapeutic outcomes, appropriate animal models are necessary. Pigs have been extensively used as valuable large animal models in biomedical research. In this review, we highlight the advantages of pig models in terms of ear anatomy, inner ear morphology, and electrophysiological characteristics, as well as recent advancements in the development of distinct genetically modified porcine models of hearing loss. Additionally, we discuss the prospects, challenges, and recommendations regarding the use pig models in HHL research. Overall, this review provides insights and perspectives for future studies on HHL using porcine models.

Keywords: Pigs; Animal models; Hereditary hearing loss; Genetic modification; Inner ear

INTRODUCTION

Hearing loss is a global health challenge that not only deteriorates quality of life but also inflicts a huge societal and economic burden. According to the World Health Organization, over 5% of the world's population, accounting for approximately 430 million people, have disabling hearing loss, with this number expected to escalate to nearly 700 million by 2050 (Babanejad et al., 2022). Hearing loss can impair a person's communication, cognition, education, and employment, seriously disrupting their normal life, and is estimated to cost $980 billion globally each year in terms of health care, educational support, and productivity loss.

Hearing loss may result from a variety of factors, including genetics, aging, loud noise, infection, physical trauma, and ototoxic drug exposure (Wang & Puel, 2018). Hereditary hearing loss (HHL), a specific form of genetically induced hearing impairment, can manifest either congenitally or progressively. HHL ranks as one of the most prevalent sensory deficits affecting humans, with a reported incidence rate of one in every 500 newborns (Nicolson, 2021). This condition is characterized by a diverse array of mutations across more than 150 genes (Kremer, 2019). Based on the presence or absence of symptoms in organs other than the auditory system, HHL is classified into syndromic hearing loss (SHL) and non-syndromic hearing loss (NSHL). NSHL is further characterized by its mode of inheritance, designated with the prefixes DFNA, DFNB, and DFNX for autosomal dominant, autosomal recessive, and X-linked inheritance, respectively. To date, 51 genes have been linked to autosomal dominant NSHL, including *DIAPH1*, *KCNQ4*, *GJB3*, *GJB6*, and *MYO7A*, 77 genes have been linked with autosomal recessive NSHL, including *GJB2*, *SLC26A4*, *TMC1*, *OTOF*, and *MYO15A*, and five genes have been implicated in X-linked NSHL, including *PRPS1*, *POU3F4*,

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SMPX, *AIFM1*, and *COL4A6*. Regarding SHL, it is classified into 11 major categories, with 124 related genes identified. Among these categories, Waardenburg syndrome and Usher syndrome are the most prevalent (Jiang et al., 2023).

Traditional interventions for hereditary deafness primarily involve the use of hearing aids and cochlear implants. However, these interventions do not restore or improve a patient's natural hearing ability. In recent years, gene therapy has emerged as a promising treatment option for deafness. This approach involves manipulating the expression or repairing mutated genes in the inner ear, leveraging high targeting ability and safety (Omichi et al., 2019). However, further advancements and refinements are required in gene editing strategies, surgical approaches, and delivery methods for deafness gene therapy (Lan et al., 2020). Thus, there is a pressing need to investigate the molecular mechanisms underlying HHL and develop novel treatment protocols to facilitate effective prevention and intervention strategies.

Animal models are indispensable tools for investigating the pathogenesis, diagnosis, and treatment of human diseases (Zhao et al., 2019), including hearing loss (Hakizimana & Fridberger, 2021). Conventional model animals for deafness research include mice (Vreugde et al., 2002), guinea pigs (Pinyon et al., 2014), and rabbits (Nguyen et al., 2023). These animal species are preferred due to their ease of breeding, handling, and genetic manipulation. Mice, in particular, are the most commonly used and numerous genetically modified mouse strains have been created that mimic various types of human hearing loss, including both non-syndromic and syndromic forms (Jiang et al., 2023). For example, the *Minar2* mutation responsible for DFNB120 was confirmed using the mouse models, with mutant mice rapidly developing progressive sensorineural deafness, accompanied by a reduction in outer hair cell stereocilia in the shortest row and degeneration of hair cells at a later age (Bademci et al., 2022). These models have significantly advanced our understanding of the mechanisms underlying hearing loss and provided valuable insights into potential therapeutic strategies for treating human hearing impairment.

Nevertheless, there are certain limitations in using mice as models for simulating human hearing loss. Discrepancies in the anatomical structure, cochlear size, and inner ear developmental process between mice and humans affect the translatability of findings from mouse models to human conditions (Guo & Yang, 2015). Notably, out of the 80 genes associated with NSHL in humans, 39 exhibit different phenotypes in mouse models (Carlson & Avraham, 2022). Therefore, more suitable experimental animals for deafness research are urgently needed.

Pigs have been widely used in the field of biomedical research due to their similar genetics, anatomy, and physiology to humans (Polejaeva et al., 2000), including in research on circulatory system diseases (Davis et al., 2014), organ transplantation (Chen et al., 2022; Niu et al., 2017), nervous system diseases (Yan et al., 2018), diabetes (Renner et al., 2013), skin diseases (Wang et al., 2019; Zhang et al., 2019), genetic diseases (Song et al., 2021; Yang et al., 2022; Zhang et al., 2017), and tumors (Wang et al., 2017). Such studies have greatly contributed to advancing basic medical research and innovative clinical technologies (Lunney et al., 2021), and porcine model is also a promising option for HHL.

In this review, we highlight the advantages of porcine models in terms of the anatomical, morphological, and electrophysiological characteristics of the inner ear, summarize current genetically modified pig models created for human hearing loss, and provide references and insights for future research in this field.

ADVANTAGES OF PORCINE MODELS IN HEARING LOSS RESEARCH

Sound waves travel through the ear canal and reach the eardrum, which vibrates and transfers sound to the ossicles in the middle ear. The ossicles amplify and transmit the sound to the cochlea in the inner ear through the oval window, causing the fluid in the cochlea to fluctuate, which stimulates the hair cells of the organ of Corti. Finally, the hair cells convert the sound vibration into nerve impulses, which are transmitted to the brain along the auditory nerve to generate the perception of hearing (Al-Sheikh & Kang, 2020).

Mice, guinea pigs, rabbits, and pigs are the most commonly used animal models for deafness research, each with their own advantages and limitations (Table 1). Despite their wide application, mice exhibit considerable differences from humans in size, lifespan, and inner ear structure and development, hindering their suitability for hearing loss research (Guo & Yang, 2015). Although guinea pigs and rabbits can address some of the drawbacks associated with HHL mouse models, they still face challenges such as limited genetic tools. In contrast, pigs exhibit a hearing range comparable to humans and naturally experience age-related hearing loss, making them valuable models for advancing our understanding of the mechanisms underlying hearing loss and the development of potential treatments for hearing-related

Table 1 Advantages and limitations of commonly used animal models of hearing loss

	Advantages	Limitations
Mice	Relatively cheap Ease of genetic modification	High-frequency hearing range differs from humans No spontaneous age-related hearing loss Surgical procedures challenging
Guinea pigs	Relatively cheap Comparable hearing range to humans Spontaneous age-related hearing loss Obvious ontological anatomical structures	Susceptibility to infections Relatively long gestation period Limited genetic tools
Rabbits	Relatively cheap Comparable hearing range to humans Spontaneous age-related hearing loss Obvious ontological anatomical structures	Relatively long gestation period Limited genetic tools
Pigs	Similarities to humans in anatomy, morphology, and electrophysiology Comparable hearing range to humans Spontaneous age-related hearing loss Accessible genetic modification	Relatively expensive and more difficult to handle Relatively long gestation period

disorders.

Similarity in temporal bone anatomy between pigs and humans

The membranous labyrinth of the inner ear is completely enclosed within the bony cavities of the temporal bone, forming a closed system essential for guiding microsurgical approaches for cochlear implantation and inner ear gene therapy. Anatomically, the temporal bone of pigs is situated in a position similar to that of humans, incorporating the squamous, mastoid, and tympanic sections, and the styloid process. The mastoid air cell system is situated medial to the temporomandibular joint and inferior to the tympanic cavity. The external ear canal is notably elongated and directed strictly upward and backward. Despite the significant variations in the placement of the mastoid and external ear canal between pigs and humans, these distinctions do not impact the pathway of otologic microsurgery or its clinical relevance (Yi et al., 2016). Furthermore, the middle ear of pigs also bears similarities to that of humans, highlighting the suitability of pigs for otologic research. Specifically, the roof, anterior, medial, and lateral walls of the pig tympanic cavity resemble those found in humans. In addition, the tympanic cavity contains the ossicular chain and facial nerve, along with its branches, closely mirroring the location and proximity of these structures in humans (Yi et al., 2014). Thus, pigs and humans exhibit high similarity in terms of the temporal bone and middle ear structure, further emphasizing the suitability of pigs as animal models for hearing research (Ji et al., 2019).

Similarity in inner ear morphology between pigs and humans

The vertebrate inner ear consists of two primary components: the cochlea and vestibular system. The cochlea transforms sound signals into nerve impulses, which are then relayed to the auditory center of the brain to generate the perception of hearing. In contrast, the vestibular system houses specialized mechanoreceptor hair cells that function as balance receptors responsible for the perception of equilibrium and balance (Magariños et al., 2012).

The cochleae of pigs and humans demonstrate notable similarities in size and structure, a contrast especially evident when compared to smaller animals like guinea pigs and mice (Figure 1A–D). The porcine cochlea features a bony canal that coils around the modiolus for 3.5 turns, closely approximating the 2.5 to 2.75 turns observed in humans. Significant structural parallels in the cochlea are also observed between the pigs and humans, particularly within the organ of Corti (Figure 1E–H). Notably, the porcine organ of Corti contains hair cells, pillar cells, Deiters cells, Hensen's cells, Bottcher cells, and inner and outer sulcus cells, mirroring those present in the spiral organ of humans (Guo et al., 2015b). A critical aspect of their similarity is that both the human and porcine cochlea are fully developed at birth, unlike the rodent cochlea, which continues to differentiate and mature after birth. Studies have revealed that the differentiation of sensory epithelial cells within the porcine cochlea initiates at the basal turn, progresses through the middle of the cochlear duct, and gradually extends to the apical turn. During the embryonic period, both pigs and humans undergo similar developmental trajectories in cochlear maturation (Guo & Yang, 2015). Together, these parallels provide a robust physiological foundation for the exploration of deafness therapies using porcine models.

Similarity in inner ear electrophysiology between pigs and humans

The auditory capabilities and electrophysiological characteristics of pigs also closely mirror those of humans. From birth, pigs possess hearing abilities within a threshold of approximately 25–30 dB SPL, slightly higher than that of humans and rodents. The frequency range for hearing in humans spans from 0.02 to 20 kHz, with peak sensitivity at 1 kHz. In contrast, pigs demonstrate a broader frequency range for hearing, reaching up to 48 kHz, with peak sensitivity at approximately 2 kHz. Rodents, on the other hand, can perceive much higher frequencies, extending beyond 100 kHz. Thus, pigs exhibit a hearing threshold and sensitivity range more in alignment with humans than with rodents (Guo et al., 2010). Furthermore, previous research has indicated that the waveform and latency of the auditory brainstem response in pigs closely resembles those observed in humans. Notably, the waveforms in both species can be differentiated into seven distinct waves, in contrast to rodents, which can only be differentiated into waves I–V. These observations suggest that pigs and humans share comparable nerve nuclei along the

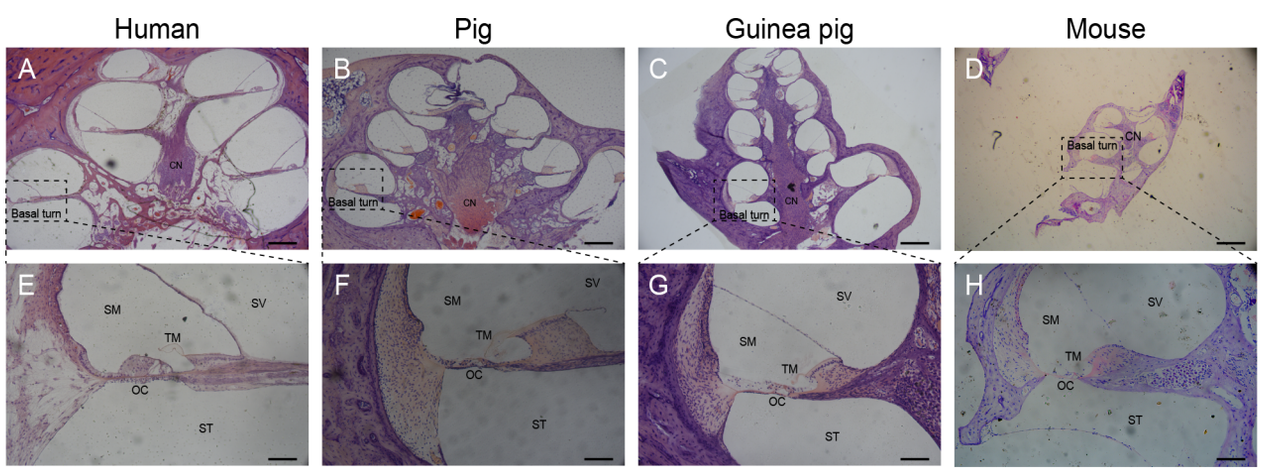

Figure 1 Hematoxylin and eosin (H&E) labeling of human, pig, guinea pig, and mouse cochlear sections, with similarities found between pigs and humans in terms of morphological structure

A–D: Mid-modiolar cochlea sections. Scale bar: 500 μm. CN: Cochlear nerve. E–H: Organ of Corti of cochlear basal turn. Scale bar: 100 μm. SV: Scala vestibuli; SM: Scala media; ST: Scala tympani; TM: Tectorial membrane; OC: Organ of Corti.

entire auditory conduction pathway, unlike mice, which lack some of these neural components (Guo et al., 2015a).

GENETICALLY MODIFIED PORCINE MODELS OF HHL

Genetically modified models have revolutionized research into human diseases by offering valuable insights into their underlying mechanisms, progression, and potential treatments (Perleberg et al., 2018; Yao et al., 2016; Zhang et al., 2022). Various genetically modified porcine models for deafness have been developed based on N-ethyl-N-nitrosourea (ENU) mutagenesis and nuclease-mediated genome editing (Table 2), with the expression of pathogenic genes associated with HHL in various cell types of the inner ear (Figure 2). The subsequent section explores established genetically modified porcine models that mimic HHL diseases.

Waardenburg syndrome

Waardenburg syndrome is characterized by four distinct clinical features: abnormal iris pigmentation, sensorineural hearing loss, inner canthus displacement, and a white forelock (Chen et al., 2016; Pingault et al., 2010). The global prevalence of Waardenburg syndrome is estimated to be about 1 in 42 000 individuals (Kumar & Rao, 2012). This condition is classified into four types, distinguished by the diverse clinical manifestations observed in patients. Its diverse pathogenic genes, including *PAX3*, *MITF*, *WS2B*, *WS2C*, *SNAI2*, and *SOX10* (Table 3), are integral for the development and migration of neural crest cells during embryonic development. Currently, several porcine models for Waardenburg syndrome have been established.

WS2A is a dominant hearing loss syndrome caused by mutations in the *MITF* gene. However, *MITF* mutations in mouse models exhibit a recessive inheritance pattern of WS2A (Tassabehji et al., 1995), complicating the study of its pathogenesis. A significant discovery in a naturally mutated pig family was a biallelic mutation in the *cis*-regulatory element

Table 2 Porcine models of HHL

Disease	Phenotype MIM number	Gene	Inheritance	Reference
WS, type 2A	193510	*MITF*	AD	Chen et al., 2016; Hai et al., 2017b
WS, type 2E	611584	*SOX10*	AD	Hai et al., 2017a
Piebaldism	172800	*KIT*	AD	Xu et al., 2020
USH, type 1C	276904	*USH1C*	AR	Grotz et al., 2022
DFNA67	616340	*OSBPL2*	AD	Yao et al., 2019
DFNA36	606705	*TMC1*	AD	Unpublished

WS: Waardenburg syndrome; USH: Usher syndrome; AD: Autosomal dominant; AR: Autosomal recessive.

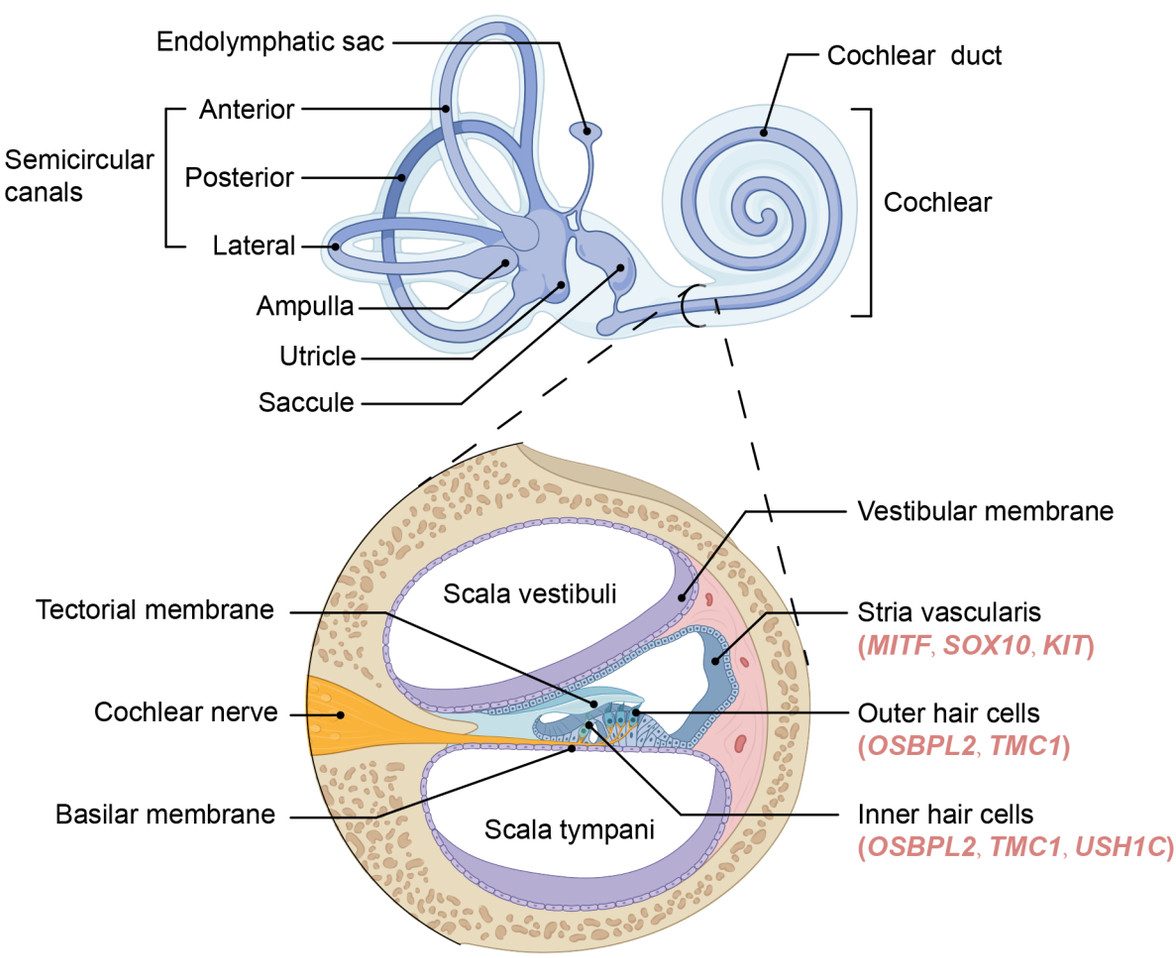

Figure 2 Causative genes of existing pig models of HHL and their expression in specific cell types of the inner ear

Table 3 Causative genes and inheritance of Waardenburg syndrome subtypes

Type	OMIM	Gene	Location	Inheritance	References
Type 1 (WS1)	193500	*PAX3*	2q36.1	AD	Ishikiriyama, 1993; Tsukamoto et al., 1992
Type 2A (WS2A)	193510	*MITF*	3p13	AD	Tachibana et al., 1996
Type 2B (WS2B)	600193	*WS2B*	1p21-p13.3	AD	Pingault et al., 2010
Type 2C (WS2C)	606662	*WS2C*	8p23	AD	Selicorni et al., 2002
Type 2D (WS2D)	608890	*SNAI2*	8q11	AR	Sánchez-Martín et al., 2003
Type 2E (WS2E)	611584	*SOX10*	22q13.1	AD	Barnett et al., 2009; Bondurand et al., 2007; Sznajer et al., 2008
Type 2F (WS2F)	619947	*KITLG*	12q21.32	AR	Vona et al., 2022
Type 3 (WS3)	148820	*PAX3*	2q36.1	AD, AR	Song et al., 2016
Type 4A (WS4A)	277580	*EDNRB*	13q22.3	AD, AR	Syrris et al., 1999; Verheij et al., 2002
Type 4B (WS4B)	613265	*EDN3*	20q13.32	AD, AR	Hofstra et al., 1996
Type 4C (WS4C)	613266	*SOX10*	22q13.1	AD	Pingault et al., 2002

AD: Autosomal dominant; AR: Autosomal recessive.

of the *MITF* gene, which eliminated the expression of the *MITF-M* isoform, leading to early degeneration of the intermediate cells of the cochlear stria vascularis, profound hearing loss, and depigmentation, resembling the typical phenotypes of WS2A observed in humans, albeit with a different inheritance pattern (Chen et al., 2016). We hypothesize that the *MITF* mutation in this porcine family occurs within the regulatory region governing gene expression, diverging from the point mutations commonly seen in human WS2A, which alter amino acid coding or lead to premature termination of mRNA translation, thereby resulting in a different inheritance pattern compared to that observed in humans. Previous studies have also successfully established a porcine model carrying the L247S mutation in exon 8 of the *MITF* gene using ENU mutagenesis, resembling the inheritance pattern and clinical abnormalities observed in human WS2A patients (Hai et al., 2017b). Pigs carrying a heterozygous mutant form of the *MITF* gene display hearing loss and reduced pigmentation in the skin, hair, and iris, as well as vascular regression and cochlear resting potential loss, mirroring the characteristics found in human patients. Notably, pigs carrying a homozygous mutant form of the *MITF* gene exhibit anophthalmia, a condition similar to that presented in human patients, further highlighting the role of *MITF* in eye development, while mice with a homozygous *MITF* mutation only develop microphthalmia (Hodgkinson et al., 1993). Research has also highlighted the effectiveness of genome editing as a therapeutic approach for treating monogenic disorders, demonstrating the correction of multiorgan abnormalities in WS2A model pigs by precisely targeting and rectifying mutations using CRISPR/Cas9 technology (Yao et al., 2021). Collectively, the establishment of porcine models for hearing loss and investigations into gene therapy approaches for this condition have yielded valuable insights into the pathogenesis of human hearing loss, opening potential avenues for novel therapeutic strategies.

Researchers have also developed a Waardenburg syndrome porcine model carrying the *KIT* c.2418T>A point mutation with autosomal dominant inheritance (Xu et al., 2020). This model exhibits severe congenital bilateral sensorineural deafness, accompanied by abnormal skin and iris color. Mechanistically, the *KIT* mutation impedes the migration and differentiation of neural crest cells to the cochlea, resulting in the loss and dysfunction of intermediate cells in the vascular pattern and changes in the inner environment of the cochlea, ultimately leading to hair cell apoptosis (Xu et al., 2020). Such findings offer a potential tool for subsequent gene therapy and clinical interventions.

Mutations in the *SOX10* gene, a crucial transcription factor, have been implicated in two distinct human syndromes: WS2E and WS4C (Zhang et al., 2012). Previous studies have successfully established a porcine model carrying a *SOX10* gene mutation using ENU mutagenesis (Hai et al., 2017a), characterized by common pigmentary abnormalities in hair, skin, and eyes, along with profound sensorineural hearing loss, as seen in WS2 patients, and cochlear malformations (Hao et al., 2018). The cochlea in the inner ear of mutant pigs presents with only 1.5 turns, displaying characteristics like fusion of the top loop, a shortened modiolus, and inhibited growth, accurately mirroring the pathological phenotype of human Mondini dysplasia. Notably, Mondini dysplasia, a congenital otic capsule malformation due to incomplete development during the seventh week of fetal life, is characterized by bony and membranous abnormities of the inner ear and a typically shortened and flattened cochlea with fewer turns. The above model represents the first established inheritable animal model of human Mondini dysplasia.

Usher syndrome

Usher syndrome is a genetically heterogeneous autosomal recessive inherited disease, featuring congenital sensorineural deafness and progressive retinitis pigmentosa. Existing rodent models of Usher syndrome mirror defects in the human inner ear but only display very mild retinal phenotypes (El-Amraoui & Petit, 2014). A pig model of Usher syndrome type 1C was successfully generated by introducing human patient homologous sequences into the *USH1C* gene locus of the pig genome (Grotz et al., 2022). This model accurately replicates various characteristics of Usher syndrome type 1C, including hearing defects, vestibular dysfunction, and visual impairment. Fibroblasts from USH1C pigs or USH1C patients contain significantly elongated primary cilia, confirming Usher syndrome type 1C as a general ciliopathy. The development of this *USH1C* pig model has substantially improved our molecular understanding of the disease, and the promising results from exploratory gene therapy experiments suggest a broad therapeutic opportunity for individuals with Usher syndrome type 1C.

DFNA67

Mutations in the *OSBPL2* gene, which encodes a protein found in stereocilia of both inner and outer hair cells, can lead to DFNA67. Using CRISPR/Cas9 technology, researchers successfully generated a porcine model with targeted

OSBPL2 gene deletion to study the function of *OSBPL2* and the molecular pathogenesis of DFNA67 (Yao et al., 2019). This model revealed progressive hearing loss in mutant pigs, accompanied by cochlear hair cell degeneration, abnormal hair cell stereocilia morphology, and hypercholesterolemia. The auditory brainstem response (ABR) threshold of 2-month-old mutant pigs (40–50 dB SPL) was slightly higher than that of wild-type pigs, while 12-month-old mutant pigs fed either basic chow or high-fat diets were completely deaf, with stimulus intensities reaching 90 and 105 dB SPL, respectively. Thus, this model provides theoretical guidance and animal resources for further exploration of gene therapy and hearing restoration for individuals with genetic hearing loss.

DFNA36

The *TMC1* gene is commonly associated with hereditary deafness, with autosomal dominant mutations leading to DFNA36 deafness. Model mice harboring a dominant *Tmc1* p.M412K (c.1235T>A) mutation, induced by chemical mutagenesis, exhibit progressive hearing loss and cochlear hair cell degeneration. Several groups have used this model to develop gene therapy strategies to enhance the survival of hair cells and attenuate hearing loss in mice (Gao et al., 2018; György et al., 2019; Yoshimura et al., 2019). However, the clinical extrapolation of gene therapy findings from mice to humans is hampered by the considerable differences in inner ear physiology and anatomy between the two species (Jiang et al., 2023). Consequently, the establishment of a human DFNA36 porcine model holds significant promise for advancing the treatment of DFNA36 and enhancing precision therapy for hereditary deafness. Our group has developed a porcine model with a *TMC1* mutation and demonstrating progressive hearing loss (unpublished data).

CONCLUSIONS AND FUTURE PERSPECTIVES

The physiological, morphological, and molecular similarities between pigs and humans have significantly propelled the utilization of porcine models for investigating pathogenic mechanisms, developing therapeutic approaches, and facilitating the translation from basic science to clinical application. Porcine models offer the ability to accurately assess the impacts of various deafness types on auditory function and cognitive abilities, establishing more objective standards for the diagnosis and classification of deafness. These models are instrumental in evaluating and optimizing auxiliary hearing devices, such as hearing aids and cochlear implants, and providing more personalized solutions for deafness improvement. Furthermore, advancements in gene editing tools have positioned gene therapy for HHL as a research hotspot for the restoration and reconstruction of auditory functions. Indeed, large animal models of hearing loss are pivotal in accelerating the development of new therapeutic solutions. Porcine models are poised to expand the therapeutic options and possibilities for the treatment and rehabilitation of deafness.

Porcine models have emerged as important tools for gaining deeper insights into the mechanisms, influencing factors, and genetic underpinnings of deafness and for providing more effective strategies for early prevention and intervention. However, establishing these models presents certain challenges. The absence of readily available inbred pig lines leads to unstable phenotypes and large individual differences in experimental pigs. Moreover, there is a need for the advancement of phenotypic analysis platforms tailored for large animals, including surgical, behavioral, and cognitive function assessments. Compared to other model animals, pigs require the establishment of well-characterized physiological health indicators and the creation of a dedicated professional database for these metrics. In addition, there is a need for optimized surgical procedures and delivery strategies for gene therapy approaches based on pig hearing loss models.

In conclusion, porcine models offer unique advantages and broad prospects in HHL research, providing an important platform and tool for basic and clinical otological studies.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

X.W., T.X.L., and Y.Z. wrote and edited the manuscript. A.L.C. and Y.F.W. contributed perspectives and conducted proofreading. L.W.X., S.L.Y., W.W.G., and S.M.Y provided photos of cochlear sections. J.G.Z. conceived and performed the final editing of the manuscript. All authors read and approved the final version of the manuscript.

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