Introduction

Sensorineural hearing loss (SNHL) is the most common sensory disorder, resulting in a major public health burden [1,2]. Epidemiological data reveal that SNHL occurs in roughly 1 out of every 500 neonates globally [3-5]. According to the World Health Organization, over 430 million people (more than 5% of the world’s population) are suffering from hearing impairment [6]. The pathogenesis of SNHL involves multiple etiologies, including genetic factors, congenital infections, noise exposure, ototoxic medications, and autoimmune/autoinflammatory disorders [7]. Specifically, genetic etiologies have been identified in approximately 50%–60% of cases of hearing impairment in children, with a high Mendelian genetic contribution [8]. SNHL exhibits significant genotypic and phenotypic heterogeneity [9]. To date, non-syndromic SNHL has been linked to 148 disease-causing genes (https://hereditaryhearingloss.org/, updated on 2024 Feb 22) while over 700–800 genes have been reported as associated with SNHL, albeit not yet been confirmed as causative. Furthermore, hearing impairment is not a disease diagnosis, but rather just a symptom. The phenotypic spectrum of hearing impairment ranges from NSHL to various forms of syndromic SNHL (s-SNHL). s-SNHL accounts for about 30% of genetic hearing loss cases, with over 400 unique syndromic conditions associated with hearing impairment identified [10]. Considering the homologous genomic sequences, anatomy, and functions of the inner ear and central auditory pathway between...
mice and humans, studies on mouse genetics have significantly deepened our insights into the molecular mechanisms operative in the inner ear [11]. Through this, a framework of deafness genes based on molecular mechanisms of inner-ear function and spatiotemporal expression has been suggested: 1) hair bundle development and function, 2) synaptic transmission, 3) cell-cell adhesion and maintenance, 4) ion homeostasis, 5) extracellular matrix, 6) oxidative stress and mitochondrial defects, and 7) transcriptional regulation [3]. The elucidation of molecular pathways in the inner ear, in conjunction with their associated phenotypes, holds the potential for the development “new normal” targeted therapies of SNHL to come.

**Evolution of Genetic Sequencing for SNHL**

Given the genotypic and phenotypic heterogeneity of SNHL, conventional targeted sequencing, such as Sanger sequencing, limits the completion of genetic diagnosis [12]. Since the 2010s, next-generation sequencing (NGS) technologies have revolutionized the genomic architecture of SNHL thanks to its capacity for simultaneous high-throughput genetic loci screening, with a focus on monogenic forms of deafness [13]. In real-world practice, genetic diagnosis of SNHL is often performed using targeted NGS sequencing methods, such as targeted-panel sequencing (TPS). This technique predominantly analyzes the coding regions of known deafness genes, with implementations such as OtoSCOPE and the Otogenetics Deafness Gene Panel [14,15]. Through TPS, the reported diagnostic yield of hearing loss varies, ranging from 12.7% to 64.3% [16]. With the significant reduction in sequencing costs, the clinical application of whole-exome sequencing and whole-genome sequencing (WGS) is becoming more practical. These methods theoretically harbor a higher capacity to detect a broader spectrum of genomic variants relative to targeted approaches [17,18], thereby accelerating the discovery of novel deafness genes and decreasing the diagnostic odyssey. In the coming years, WGS, moving beyond the exome era, will enable the detection of diverse classes of variants, such as non-coding, regulatory, and structural variations, thereby expanding our understanding of SNHL genetics. Consequently, achieving genetic completion, coupled with comprehensive genome-phenome landscape, is crucial because this information can offer clinical implications of SNHL clinical practice, including genotype/mechanism-based targeted therapies, referral to specialists, precision auditory rehabilitation, or reproductive counseling with preimplantation genetic testing for next babies.

**Targeted Small Molecules for Genetic Hearing Loss**

Small molecular compounds are currently under investigation in clinical trials for their potential therapeutic effects on hearing loss. Autoimmune/autoinflammation serves as one of the classifications in the pathogenesis of SNHL [19], thus identifying small molecules that regulate autoimmune/autoinflammation could serve as hearing loss therapeutics. Gain-of-function variants in NLRP3 lead to the aberrant activation of the NLRP3 inflammasome [20], causing disease spectrum of cryopyrin-associated periodic syndromes including non-syndromic autosomal dominant hearing loss (DFNA34) [21]. This complex operates as an intracellular sensor of innate immunity, with a predominant expression in immune cells such as monocytes and macrophages, orchestrating inflammatory responses [22,23]. Activation of the NLRP3 inflammasome, in turn, initiates the cleavage of procaspase-1 to its active form, caspase-1, which subsequently converts pro-IL-1β into mature IL-1β. The cytokine IL-1β, a key mediator of autoinflammation, is also a target for therapeutic interventions. In mouse cochlea, activation of NLRP3 inflammasome in resident macrophage/monocyte-like cells leads to the secretion of IL-1β [20,24]. Since the first study by Nakanishi, et al. [20] in 2017, there have been several reports that IL-1β blockade therapy can elicit the reversal or improvement of autoinflammatory hearing loss caused by gain-of-function variants in NLRP3 [25-27].

In addition, certain variants in deafness genes can precipitate inner ear proteinopathies, characterized by abnormal protein aggregation and accumulation in the inner ear. Addressing this, therapeutic avenues may involve the enhancement of proteolytic pathways to facilitate protein degradation. A pertinent example is the oxysterol binding protein-like 2 (OSBPL2), encoded by the human OSBPL2 gene. This protein is part of a family akin to the oxysterol-binding proteins (OSBPs), which are pivotal in lipid transport and metabolism within cells [28]. The OSBP family is instrumental in lipid-mediated signal transduction, the preservation of intracellular lipid reserves, and the orchestration of lipid translocation across distinct membrane domains [29]. Notably, the OSBPL2 is expressed in both inner and outer hair cells, and disease-causing variants in OSBPL2 have been linked to non-syndromic autosomal dominant hearing loss (DFNA67) [30]. The cellular accumulation of a truncated OSBPL2 mutant proteins harboring loss-of-function alleles has been observed, which disrupts autophagy-lysosomal pathway critical for protein degradation [31]. Upon the cellular mechanism, we hint that the aggregated mutant protein is amenable to degradation.

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through the application of rapamycin, a known autophagy enhancer [31]. The hearing loss in transgenic mouse models expressing the mutant OSBPL2 protein was observed but improved with rapamycin treatment [31]. Similarly, rapamycin partially ameliorated hearing loss and tinnitus in DFNA67 patients [31]. In addition to OSBPL2-related DFNA67, various genetic variants that can induce inner ear proteinopathy have been identified, raising anticipation for the development and clinical application of target molecules.

**Progress in Inner Ear Gene Therapy**

Gene therapy is a field of research aimed at treating diseases by modifying, replacing, or repairing abnormal genes [32]. This field is particularly relevant to genetic hearing loss, where a deep understanding of phenome/genome landscape and advancements in gene delivery methods are driving rapid progress. A primary strategy in gene therapy involves gene replacement or augmentation, which introduces a functional gene copy to substitute a deficient or null gene and restore its activity, especially in conditions characterized by loss-of-function [33,34]. For example, variants in the OTOF are known to cause auditory neuropathy (DFNB9) [35]. OTOF encodes otoferlin, a protein localized primarily to the ribbon synaptic vesicles in cochlear inner hair cells, playing a crucial role in calcium-dependent synaptic vesicle exocytosis at ribbon synapses [36]. Thus, defective otoferlin in DFNB9 may hinder vesicle recycling and membrane trafficking, leading to sound dysynchrony in central auditory tracts and compromised speech and language decoding [35]. Research in mouse models deficient in OTOF has shown that cDNA delivery via adenovirus-associated virus (AAV) can reverse hearing loss [34,37-39]. The safety and systemic biodistribution of AAV1-hOTOF, under the control of the Myo15 promoter, have been further validated in primate models, demonstrating its potential applicability for therapeutic interventions in auditory neuropathy [40]. With preclinical evidence on both restoration of hearing function and safety, OTOF gene therapy has advanced into the phase of human clinical trials [41,42]. In detail, AAV-OTOF gene therapy was administered unilaterally to two pediatric patients, aged 5 and 8 years, who were diagnosed with auditory neuropathy due to OTOF biallelic variants. This treatment leads to significant hearing recovery within 3 months post-treatment, as evidenced by improvements in auditory brainstem response thresholds (ABRT) and pure-tone audiometry [41]. In a parallel study, AAV1-hOTOF gene therapy was proven to be safe and effective for pediatric patients aged 1–18 years diagnosed with auditory neuropathy caused by OTOF biallelic variants. The results observed no dose-limiting toxicities or serious adverse events, and five of the six patients exhibited significant hearing improvements, as demonstrated by improved ABRT and speech perception [42]. The early results from these tests, even though they were over a short time, have shown that the therapy could work well. Further studies and longitudinal analyses are anticipated to elucidate the long-term outcomes and safety profiles associated with this novel therapeutic approach. This paves the way of the era of inner ear gene therapy for the treatment of genetic hereditary hearing loss caused by various deafness gene variants [42].

**Revolutionizing Hearing Loss Treatment:**

The Impact of CRISPR-Cas9 Gene Editing

Clustered regularly interspaced short palindromic repeats (CRISPR)-based technologies are under development for site-specific genome editing to address several human genetic disorders. Cas9 nuclease generates DNA double-strand breaks (DSBs) at target sites, which induces the cellular repair process through either homology-directed repair or non-homologous end-joining pathways [43]. The study published in *Nature* in 2017 is the first research on inner ear gene editing using CRISPR-Cas9, showing that delivering Cas9 nuclelease in the *TMCI* Beethoven mouse model leads to the recovery of hearing loss [44]. Subsequent studies have explored the effectiveness of Cas9 nuclelease in vivo on various deafness-related autosomal dominant genes, such as KCNQ4 and MYO7A [45-47]. At the end of last year, EXACEL, a therapy based on CRISPR gene editing technology, was FDA-approved as the first gene-edited treatment for patients with β-thalassemia and sickle cell disease [48].

However, the risks associated with CRISPR-mediated DSBs, such as large DNA deletions, chromosomal depletion, and p53-driven programmable cell death, remain a critical concern [49-51]. In response, new gene editing techniques such as base editing and prime editing, which include single-strand breaks instead of DSBs, have been developed [52,53]. These techniques allow for the precise on-target editing of disease-causing genetic variants, significantly reducing the risk of off-target effects. Such advancements are expected to enhance the safety and effectiveness of CRISPR-guided gene editing in clinical applications, offering a more secure and efficient approach to gene therapy in the field of otology.

As an example of base editing, in the Baringo mouse model, which possesses the *TMCI* Y182C variant, the application of cytosine base editors and guide RNA (gRNA) through a dual AAV vector successfully amended the *TMCI* point mutation [54]. This correction led to the restoration of sensory
transduction in a majority of inner hair cells and achieved a partial enhancement in auditory function [54]. Furthermore, adenine base editing has been applied to the PCDH15 gene, where variants cause Usher syndrome type 1F (USH1F), characterized by congenital loss of hearing and balance, along with progressive visual impairment [55]. The PCDH15 R245X variant, notably common in the Ashkenazi Jewish population, is a predominant cause of USH1F [56]. A PCDH15 R245X humanized mouse model has shown promising results in hearing restoration, highlighting the potential application of base editing to rescue hearing impairment.

Conclusion

The advent of NGS significantly improved diagnosis of genetic hearing loss, leading to an era of precision medicine based on the genotype and mechanisms underlying the condition. Traditional approaches, such as panel and exome sequencing, used in clinical practice are now facing challenges due to the growing need to explore the diagnostic advantages of WGS. Identifying disease-causing variants that correspond with clinical phenotypes is crucial, especially in SNHL where therapeutic options are lacking. Small molecules and gene therapy represent good examples of how breakthroughs in genetic understanding and sequencing technologies can be translated into SNHL treatments. Remarkably, the successful outcomes of the first-in-human trial of OTOF gene therapy spotlight the potential for treating various forms of genetic hearing loss. Beyond gene therapy, we hope for a future where such conditions associated with SNHL can be effectively managed or even cured by gene editing.

Conflicts of Interest

The authors have no financial conflicts of interest.

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