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Exploring the Heterogeneity of Non-Syndromic Hearing Loss: A Comprehensive Review of Implicated Genes and the Role of Whole Exome Sequencing

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***Abstract*— Background: Hereditary hearing loss (HHL) accounts for approximately 50-60% of all hearing loss (HL) cases, highlighting the significant role genetics play in this condition. HHL can be mostly classified into two main forms: syndromic and non-syndromic. Of these, non-syndromic hearing loss (NSHL) is the most prevalent, contributing to over 70% of HHL cases. The hearing process involves a complex interplay of various components inside the ear, working together to convert sound waves into comprehensible signals for the brain. Non-syndromic hearing loss (NSHL) is known for its extraordinary diversity and complexity, influenced by a vast array of genes, with more than 120 identified thus far. These genes can be categorized into distinct functional groups, encompassing cochlear ion homeostasis, hair bundle development and cell adhesion, synaptic transmission, transcriptional regulation, and mitochondrial function. The advent of next-generation sequencing (NGS) technologies has been instrumental in unraveling the genetic underpinnings of NSHL. Among NGS techniques, whole exome sequencing (WES) appeared as a revolutionary advancement, outperforming other approaches like targeted panel sequencing and whole-genome sequencing in terms of cost-effectiveness and efficiency. WES not only aids in deciphering the genetic basis of hearing loss but also offers the potential to revolutionize patient care and diagnosis. It paves the way for a new era for precision medicine in the realm of hearing loss, promising more accurate and tailored treatments for affected individuals. This transformative approach is poised to bring about substantial advancements in our understanding of hereditary hearing loss and its clinical management.**

Keywords— HHL, NGS, WES, NSHL

I. Introduction

Hereditary hearing loss (HHL), also known as genetic hearing loss, constitutes a significant portion of hearing loss cases. It is estimated that approximately 50-60% of all hearing loss (HL) cases have genetic origins [1]. HHL can be inherited through various patterns, including autosomal dominant, autosomal recessive, X-linked, and mitochondrial inheritance [2]. This diversity in inheritance patterns adds to the complexity of HHL. In broad terms, HHL was categorized into two primary forms: syndromic and non-syndromic. These classifications help to differentiate cases where hearing loss is associated with other medical conditions (syndromic) from cases where hearing loss occurs as an isolated condition (non-syndromic). Non-syndromic hearing loss (NSHL) is the most prevalent form of hereditary hearing loss, accounting for over 70% of cases [3]. The inheritance patterns for NSHL vary, with autosomal recessive being the most common (70-80%), followed by autosomal dominant (15-20%), and rare occurrences of X-linked or mitochondrial inheritance (1-2%) [4]. NSHL was characterized by remarkable diversity and complexity; Next generation sequencing (NGS) has proven instrumental in uncovering the genetic underpinnings of NSHL and revolutionizing genetic testing.

The primary objective of this review is to present a comprehensive overview of the frequently implicated genes

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in non-syndromic hearing loss (NSHL). It aims to emphasize the remarkable genetic heterogeneity observed in NSHL and how the advent of high-throughput technologies has significantly enhanced our understanding of this diversity. In the following sections, we will commence by examining the pivotal genes that play crucial roles in hearing function. These genes will be categorized based on their specific functions, providing insights into the intricate mechanisms underlying auditory perception. Subsequently, we will delve into recent advancements in high-throughput sequencing technologies, particularly focusing on the significance of whole exome sequencing (WES) and its impact on NSHL testing. Taken together, these insights collectively underscore the complex genetic landscape of NSHL and highlight the pivotal role of high-throughput sequencing technologies, such as WES, in unraveling the genetic basis of hereditary hearing loss.

II. Common Forms of Non-Syndromic Hearing Loss (NSHL)

The process of auditory function is a highly complex and multifaceted one, relying on the coordinated efforts of numerous proteins to ensure normal hearing. The interplay of these proteins forms a complex web of mechanisms that facilitate the detection and processing of sound within the ear. The known genes associated with NSHL so far encompass over 120 genes categorized into functional groups, such as cochlear ion homeostasis, hair bundle development, synaptic transmission, transcriptional regulation, and mitochondrial function [5]. Subsequently, we will explore in detail the most prevalent and significant genes within each of these functional groups.

III. Genes involved in cochlea ion homeostasis

In the inner ear, maintaining ion homeostasis in the cochlear duct is crucial for proper signal transmission during hearing. Various mechanisms control the movement of molecules across cell membranes to uphold this balance. Key components, such as gap junctions and ion channels, play essential roles in this regulation. Mutations in genes governing these components result in hearing loss [6].

IV. Gap junctions

In the cochlea, gap junctions (GJ) are abundant and facilitate the transfer of small molecules between neighboring cells via connexins, which form hexameric structures called connexons. These connexons combine to create functional gap junctions. In the inner ear, the widespread presence of gap junctions is crucial for maintaining fluid homeostasis and enabling communication between cells [7,8].

A. GJB2 gene:

The GJB2 gene, encoding connexin 26 (Cx26), plays a critical role in maintaining normal hearing function. Cx26, a member of the connexin protein family, is expressed in various gap junctions within the inner ear. Its primary function involves facilitating the recycling of potassium ions (K+) from the cochlea endolymph, which is essential for preserving the cochlea's electrical potential and ensuring proper sensorineural hearing [9,10]. Mutations in GJB2 are a major cause of hereditary hearing loss (HHL), responsible for around 50% of autosomal recessive non-syndromic hearing loss (ARNSHL). Additionally, GJB2 mutations are also linked to autosomal dominant hearing loss (DFNA3) and certain syndromic hearing loss conditions [11].

B. GJB6 gene:

The GJB6 gene codes for Connexin 30 protein (Cx30), which plays a role in forming gap junctions between supporting cells in the cochlea. These gap junctions can also exist as hemichannels on the surface of these cells [12]. GJB2 and GJB6 genes, closely linked at the DFNB1 locus, are common genetic causes of hereditary hearing loss (HHL) globally [13]. Deficiencies in Cx30 can disrupt the intrastrial fluid-blood barrier in the cochlear stria vascularis [14]. Pathogenic variants in GJB6 can lead to both dominant and recessive non-syndromic hearing loss (NSHL), with different inheritance patterns, including mutations in GJB6 alone, GJB2 alone, or a combination of both [15].

Ion channels

C. KCNQ4 gene:

The *KCNQ4* gene codes for a voltage-gated potassium channel protein that is vital for functions like sound transmission, membrane potential maintenance, and osmotic balance [16]. Mutations in this gene can lead to autosomal dominant hearing loss (DFNA2), making up about 9% of all cases of autosomal dominant non-syndromic hearing loss (ADNSHL) [17].

D. SLC26A4 gene:

The *SLC26A4* gene codes for a membrane protein called pendrin, which regulates ion transport in the inner ear and is present in the thyroid [18]. Mutations in this gene can lead to different clinical conditions, including non-syndromic hearing loss (DFNB4), hearing loss with the enlargement of specific ear structures (EVA), and Pendred syndrome, characterized by hearing loss, thyroid enlargement (goiter), and additional symptoms [19].

V. Genes involved in hair bundle development and functioning

The proper functioning of hair cells in the auditory system relies heavily on their structural integrity. Changes in the structure of these cells can lead to various forms of hearing loss. Maintaining the structure of hair cells is controlled by a complex interplay of integral membrane proteins, adhesion proteins, ciliary proteins, and myosins [20].

VI. Integral Membrane Proteins

A. *TMC1 gene:*

The *TMC1* gene codes for a protein crucial for the function and maintenance of inner ear hair cells, playing a role in mechanosensory transduction channels [21,22]. Mutations in *TMC1* can cause autosomal dominant (DFNA36) or autosomal recessive non-syndromic hearing loss (DFNB7/B11).

B. *TMIE gene:*

The *TMIE* gene encodes a protein with two transmembrane domains, which is essential for the development, maturation, and maintenance of inner ear sensory hair cells, contributing to the hearing process [23]. Loss-of-function mutations in *TMIE* have been linked to autosomal recessive hearing loss (DFNB6) in both humans and animals [23].

VII. Ciliary structure

A. *STRC gene:*

The *STRC* gene codes for an extracellular protein called stereocilin, primarily found in the stereocilia of outer ear hair cells. Stereocilin is essential for forming connections between stereocilia and the tectorial membrane, ensuring proper positioning and cohesion of the stereociliary tips [24]. Mutations in the *STRC* gene are associated with bilateral early-onset mild-to-moderate hearing loss, known as DFNB16 [25].

VIII. Adhesion

CDH23 gene:

The *CDH23* gene codes for Cadherin-23, a calcium-dependent cell adhesion protein found in inner and outer hair cells of the cochlea. It plays a crucial role in forming "Tip Link" structures in stereocilia, essential for proper hair cell function. Mutations in *CDH23* are linked to two types of hearing loss: non-syndromic hearing loss (DFNB12) and syndromic hearing loss (Usher syndrome) [26,27].

IX. Myosin family

Myosins are molecular motor proteins that interact with actin, using ATP to generate force and facilitate movement. They consist of a head, tail, and neck region and play essential roles in various cellular processes, including actin cytoskeleton rearrangement, tension regulation of actin filaments, and organelle transport [28]. There are seven major families of myosin genes associated with different types of hearing loss: *MYO1A, MYO3A, MYO6, MYO7A, MYO15A, MYH9,* and *MYH14,* linked to non-syndromic hearing loss. These myosin's are crucial for hearing by contributing to the structure and movement of stereocilia in the inner ear, facilitating the opening and closing of ion channels, which allows the conversion of sound waves into electrical signals processed in the brain [28] [29].

X. Genes involved in Extracellular matrix (ECM)

The extracellular matrix (ECM) serves a dual role in offering structural support and regulating cell signaling. It comprises proteins like collagens, proteoglycans, and non-collagenous glycoproteins [30].

A. *TECTA gene:*

The *TECTA* gene produces the alpha tectorin protein, a significant component of the non-collagenous glycoproteins present in the tectorial membrane, which overlays the organ of Corti in the inner ear [31]. This protein's interaction with the stereocilia of specialized sensory hair cells is essential for initiating the movement of these cells during sound transmission in the cochlea [32]. Mutations in the *TECTA* gene are linked to both autosomal dominant (DFNA8/12) and autosomal recessive (DFNB21) non-syndromic hearing loss (NSHL).

B. *COCH gene:*

The *COCH* gene produces the cochlin protein, a significant component of the inner ear's extracellular matrix, primarily found in the spiral ligament and spiral limbus regions [33]. While the exact role of cochlin is not fully understood, it is believed to be involved in providing structural support, aiding in sound processing, and maintaining balance in the inner ear [34]. Mutations in the *COCH* gene are associated with autosomal dominant hearing loss (DFNA9) and, more recently, autosomal recessive hearing loss (DFNB110) [35].

XI. Genes involved in synaptic transmission

Effective transmission of auditory signals from the cochlea to the brain depends on the synapses established between hair cells and auditory nerve neurons, allowing the transformation of sound waves into electrical signals [36]. The intricate operation of these synapses is regulated by a wide range of proteins.

A. *OTOF gene:*

The Otoferlin gene (*OTOF*) produces the Otoferlin protein, which is essential for the synapse function in inner hair cells (IHCs) of the auditory system. Mutations in *OTOF* result in autosomal recessive hearing loss (DFNB9) by disrupting the release of synaptic vesicles at the IHC synapse, causing a failure in signal transmission [37].

B. *GIPC3 gene:*

The *GIPC3* gene produces a protein belonging to the GIPC family, known for its interaction with GAIP (G Alpha-interacting protein), which regulates vesicle transport in G protein-coupled signaling complexes [38]. *GIPC3* is

vital for the maturation of hair bundles in both inner and outer hair cells and supports the long-term survival of these cells and the spiral ganglion by aiding in the transport, signaling, and recycling of molecules and proteins across cell membrane [39]. Mutations in *GIPC3* are linked to autosomal recessive non-syndromic hearing loss, specifically DFNB15 [40,41].

XII. Genes involved in transcriptional regulation

Transcription factors are proteins that bind to DNA and control the process of gene transcription. They are crucial for various cellular functions, including maintenance, differentiation, development, response to neighboring cells, and environmental adaptation [42].

A. EYA4 gene:

The *EYA4* gene codes for the Eyes absent 4 protein, a member of the EYA family involved in transcriptional activation and essential for normal embryonic development [43]. *EYA4* also plays a critical role in the maturation and maintenance of the inner ear system [43,44]. Mutations in *EYA4* are a rare cause of sensorineural hearing loss, primarily associated with autosomal dominant non-syndromic hearing loss at the DFNA10 locus [45,46]. In some cases, these gene mutations have been linked to autosomal dominant syndromic hearing loss along with dilated cardiomyopathy [47].

B. POU3F4 gene:

The *POU3F4* gene produces a transcription factor with two DNA-binding domains [48,49], and while its specific target genes are not fully known, it is essential for the development of the hearing system [50]. Mutations in this gene lead to DFNX2, an X-linked recessive hearing loss, accounting for around half of all cases of X-linked hearing loss [51]. DFNX2 is characterized by progressive conductive and sensorineural hearing loss, along with distinct temporal bone abnormalities seen in radiological examinations [52].

XIII. Genes involved in mitochondrial function

Mitochondrial-related hearing loss can arise from mutations in nuclear genes that code for mitochondrial proteins or from mutations in mitochondrial DNA (mtDNA) [53]. Consequently, mitochondrial hearing loss can be inherited through Mendelian patterns or maternal inheritance. Non-syndromic hearing loss due to mitochondrial mutations is relatively rare and is mainly associated with two genes: *MT-RNR1* and *MT-TS1*.

A. MT-RNR1 gene:

The *MT-RNR1* gene, found in the mitochondrial genome, encodes mitochondrial 12s ribosomal RNA. Mutations in this gene are associated with an increased risk of aminoglycoside-induced hearing loss and maternally inherited non-syndromic hearing loss [54]. Aminoglycoside-related hearing loss typically occurs shortly after exposure to these antibiotics, and the m.1555A>G variant in *MT-RNR1* is strongly linked to this condition [55].

B. MT-TS1 gene:

The *MT-TS1* gene codes for mitochondrial transfer RNA for serine 1. Mutations in this gene were linked to sensorineural hearing loss (SNHL), usually in a non-syndromic context. These mutations are thought to hinder tRNA processing, leading to a decline in cellular oxidative phosphorylation [56]. However, specific *MT-TS1* variants have been associated with syndromic cases. For instance, the m.7445A>G variant is found in families with hearing loss and palmoplantar keratoderma, while the m.7512A>G variant is linked to various forms of MERRF syndrome [57,58].

XIV. Whole exome sequencing and its application in non-syndromic hearing loss

A. The Importance of Genetic Testing in Non-Syndromic Hearing Loss (NSHL)

Understanding the genetic causes of hereditary hearing loss holds profound significance for several critical aspects of healthcare and research. Firstly, it provides essential insights into the fundamental mechanisms and underlying pathology of this condition. This knowledge not only helps us comprehend how hearing loss occurs at a genetic level but also paves the way for potential breakthroughs in treatment and prevention strategies. Secondly, elucidating the genetic basis of hearing loss is indispensable for early diagnosis. Identifying the specific genetic mutations responsible for hearing loss allows the healthcare professionals to diagnose individuals at risk earlier stage, enabling timely interventions and tailored management plans [59,60]. Furthermore, this genetic understanding serves as a cornerstone for the development of more effective treatment regimens. With insights into the genes and pathways involved, researchers can explore targeted therapies that have the potential to ameliorate or even reverse hearing loss. Lastly, improved insights into the genetic causes of hearing loss enhance the quality of genetic counseling services provided to affected individuals and their families. It allows for more accurate risk assessment, informed decision-making, and family planning [59,61-63].

Traditional genetic testing methods like Sanger sequencing are currently the global standard for variant detection through sequencing. However, they have limitations for high-throughput applications, including issues with read length, runtime, and per base cost [64]. Thankfully, a powerful high-throughput technology called next-generation sequencing (NGS) or massively parallel

sequencing (MPS) has emerged in the last decade. This groundbreaking sequencing method can efficiently sequence millions of small fragments spanning the entire genome or specific regions of interest, such as the coding part of the genome (exome), at a more cost-effective price and in less time compared to Sanger sequencing [65,66].

B. Whole Exome Sequencing (WES)

Whole Exome Sequencing (WES) is a Next-Generation Sequencing (NGS) technique designed specifically to sequence the protein-coding regions, known as exons, within the genome. These exonic regions encompass roughly 22,000 genes, constituting 2% of the entire genome. Remarkably, a substantial 85% of disease-associated genetic variants are primarily found in these exonic regions. This highlights the cost-effectiveness and efficiency of WES compared to whole-genome sequencing, especially in unraveling the genetic basis of conditions marked by significant genetic diversity, such as HHL [66].

The process of WES involves enriching the exonic DNA through the use of molecular baits or probes. This is followed by high-throughput sequencing and sophisticated bioinformatics analyses, which enable the identification of various genetic variations beyond single nucleotide variants (SNVs). These variations encompass insertions, deletions, and copy-number variations (CNVs). As a result, Whole Exome Sequencing has emerged as a pivotal approach in modern genomic research, offering comprehensive insights into the genetic factors underlying complex disorders, such as NSHL [63].

C. The Application of Whole Exome Sequencing (WES) in Non-syndromic Hearing Loss (NSHL)

Hereditary hearing loss (HHL) is marked by significant genetic diversity, with over 120 genes linked to Non-Syndromic Hearing Loss (NSHL) (https://hereditaryhearingloss.org/). This extensive genetic heterogeneity highlights the complexity of unraveling the genetic basis of hearing loss [67]. Fortunately, recent advancements in Next-Generation Sequencing (NGS) have transformed our capacity to address this genetic diversity. In just a few years, NGS technologies have unveiled over a quarter of all known NSHL-related genes [68]. This rapid progress highlights the potential of NGS to transform our understanding of hereditary hearing loss. It's essential to recognize that our current understanding is likely far from complete. Research suggests that approximately 1% of all human genes are linked to hearing, indicating that there is still a vast landscape of hearing loss-related genes waiting to be discovered and explored [69].

In the past, the identification of pathogenic variants associated with hearing loss, aside from common genes like *GJB2* and *SLC26A4*, proved exceedingly challenging. Conventional DNA sequencing methods, such as Sanger sequencing, were employed but made comprehensive genetic testing for hearing loss impractical [70]. This is where NGS technology has truly shone. NGS enables the simultaneous sequencing of multiple genomic regions, making it particularly well-suited for genetically heterogeneous conditions like hereditary hearing loss. Targeted NGS panels, which focus on known hearing loss genes, have become popular for diagnosing hearing loss due to their cost-effectiveness and ease of analysis. However, they are constrained by our existing knowledge of hearing loss genes. On the other hand, Whole Exome Sequencing (WES) has emerged as a powerful tool in the quest to uncover the genetic basis of hereditary hearing loss. Unlike targeted panels, WES eliminates the need for the continual development and validation of customized panels. More significantly, WES has the potential to identify mutations in known hearing loss genes while also uncovering novel genes associated with hearing loss. This makes WES an invaluable approach for conducting comprehensive genetic analyses in individuals affected by hearing loss, ultimately leading to a deeper understanding of the genetic basis of hereditary hearing loss (HHL) [71].

XV. Conclusions

Auditory function is very complex. Non-syndromic hearing loss is characterized by extraordinary genetic diversity, with researchers continually identifying new associated genes. To date, the count has surpassed 120 genes, and the list continues to expand. Understanding this genetic heterogeneity is crucial in deciphering the underlying causes of non-syndromic hearing loss. Each of these genes may contribute to hearing loss in distinct ways, affecting different aspects of auditory function. As we delve deeper into the genetic intricacies of hearing loss, we gain valuable insights that can inform research, diagnosis, and treatment strategies for individuals affected by this condition. WES has gained widespread acceptance in both clinical practice and research, emerging as a powerful tool for investigating hereditary hearing loss. Its ability to identify patient-specific causes in genetically diverse disorders or undiagnosed Mendelian phenotypes has the potential to revolutionize patient care. Among the various conditions, hereditary hearing loss (HHL) is particularly well-suited for harnessing this cutting-edge technology. WES should be strongly considered, especially in cases where traditional single-gene testing fails to provide a conclusive diagnosis or when it offers a quicker and more cost-effective route to precise genetic diagnosis. In summary, WES represents a transformative approach that not only aids in unraveling the genetic basis of hearing loss but also holds promise for enhancing patient care and diagnosis, ushering in a new era of precision medicine for HL.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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