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CASE REPORT

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Mutation Detection in *MYO15A* Gene in an Iranian Family with Non-Syndromic Hearing Loss

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Abstract

Hearing loss, recognized as one of the most prevalent sensory disorders, encompasses both syndromic and non-syndromic manifestations, with the identification of 87 genes and over 100 genetic loci in autosomal recessive non-syndromic hearing loss marking significant progress in understanding its genetic basis. In this case report, we showcase a non-syndromic hearing loss scenario involving a 21-year-old man experiencing progressive hearing loss. Through whole-exome sequencing, we unveiled a previously unreported homozygous mutation, c.1178_1179delAC; p.Tyr393Serfs*38, located in exon 2 (NM_016239.4) of the *MYO15A* gene in the proband. The newly identified mutation, causing a new reading frame (p.Tyr393Serfs*38), results in an early encounter with a stop codon, leading to the formation of a shortened protein. These findings advance our understanding of the molecular mechanisms involved in autosomal recessive non-syndromic hearing loss, contributing to broader scientific knowledge and potential breakthroughs in hearing loss research.**(International Journal of Biomedicine. 2024;14(1):165-169.)**

Keywords: hearing loss • *MYO15A* gene • mutation

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Abbreviations

AD, autosomal dominant: **AR**, autosomal recessive; **ARNSHL**, autosomal recessive non-syndromic hearing loss; **HL**, hearing loss; **mt**, mitochondrial; **NSHL**, non-syndromic hearing loss; **NGS**, next-generation sequencing; **WES**, whole-exome sequencing; **XL**, X-linked; **YL**, Y-linked.

Introduction

Hearing loss (HL) stands as one of the most pervasive sensory disorders, exerting a substantial impact on an individual's quality of life. This condition manifests in diverse forms, both syndromic and non-syndromic, exhibiting an array of inheritance patterns that contribute to its complexity. The inheritance patterns include autosomal dominant (AD), autosomal recessive (AR), Y-linked (YL), X-linked (XL), and mitochondrial (mt), each presenting unique challenges and considerations in understanding and addressing the underlying causes of hearing impairment.[1–3] Autosomal recessive non-syndromic hearing loss (ARNSHL) is particularly common and constitutes approximately 80% of NSHL cases.[4] To date,

87 genes and over 100 genetic loci associated with ARNSHL have been documented, as detailed in the hereditary HL database (http://hereditaryhearingloss.org/).

ARNSHL is frequently associated with mutations in key genes that play a pivotal role in auditory function. Among these genes, several stand out as particularly significant contributors to ARNSHL, each encoding crucial proteins involved in the intricate mechanisms of hearing. The genes commonly implicated in ARNSHL include myosin XVA (*MYO15A*, MIM#602666), cadherin-related 23 (*CDH23*, MIM#605516), solute carrier family 26 member 4 (*SLC26A4*, MIM#605646), transmembrane channel-like 1 (*TMC1*, MIM#606706), gap junction protein beta 2 (*GJB2*, MIM#121011), and otoferlin (*OTOF*, MIM#603681). Remarkably, each of these genes has exhibited a remarkable diversity of mutations, with more than 20 different variants identified. Intriguingly, the majority of these mutations have been identified in consanguineous families, suggesting a complex interplay of genetic factors within closely related individuals.[5]

The prevalence of numerous genes implicated in hearing impairment contributes to the heterogeneity of HL, highlighting the need for more efficient approaches in routine genetic testing and comprehensive genetic analysis. Whole-exome sequencing (WES), a technique based on next-generation sequencing (NGS) platforms, has revolutionized the discovery of causative genes and diagnoses for heterogeneous inherited disorders.[1, 6–9] WES stands as a valuable method, offering a thorough and efficient approach to pinpoint causative mutations in individuals with genetic disorders. This advanced technique allows for the targeted sequencing of the coding regions of genes, enabling rapid, accurate, and cost-effective identification of mutations underlying single-gene disorders.

In societies marked by a high rate of consanguinity, such as Iran with approximately 40%, Hereditary HL holds special significance, providing a pathway for rare pathogenic mutations to manifest. HL emerges as the second most prevalent disability within the Iranian population, representing a significant health concern. The prevalence of HL in Iran underscores the need for a comprehensive understanding of its etiology and the factors contributing to its occurrence. One notable aspect contributing to the dynamics of HL in Iran is the prevalence of consanguineous marriages within the population. Consanguinity, or marriage between close relatives, is a cultural practice that has been historically prevalent in Iran. It is anticipated that within the Iranian HL population, the rate of consanguineous marriages could reach as high as 65%.[10]

Based on this evidence, our objective was to identify defective genes associated with NSHL in an Iranian family using WES. This study reveals a novel chr17-18023291 TAC>T mutation in the *MYO15A* gene, demonstrating a pathogenic effect that could potentially explain the NSHL phenotype observed in this specific Iranian family.

Case Presentation

We present the case of a 21-year-old male, the only son offspring of an Iranian consanguineous couple, as illustrated in Figure 1. The patient's medical history is noteworthy for a diagnosis of congenital HL, with no apparent dysmorphic features detected upon clinical examination. The familial context is particularly significant, as there is no substantial history of HL within the family unit. Both parents share a consanguineous relationship, which adds a layer of complexity to the genetic considerations in this case.

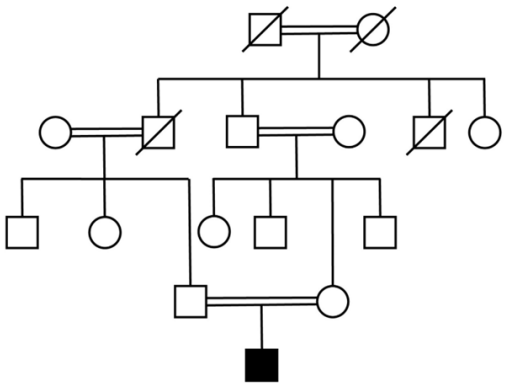

Fig. 1. *Pedigree of the studied family. Symbols marked by a slash indicated that the subject was deceased. Males were indicated by squares; females were indicated by circles. The blackened symbol represents the proband.*

In addition to the absence of a familial HL history, the patient's medical background is marked by the lack of any documented systemic diseases. This absence of systemic health issues further focuses the investigation on the genetic underpinnings of the congenital HL observed in the patient. Given the genetic implications associated with consanguinity, our objective is to explore and characterize the molecular basis of congenital HL in this unique case. To achieve this, we plan to employ advanced genetic testing methodologies, including WES, to unravel the potential genetic mutations or variants contributing to the patient's hearing impairment.

As we delve into the intricacies of this case, the aim is to not only provide a comprehensive understanding of the patient's condition but also to contribute valuable insights into the broader genetic landscape of congenital HL within consanguineous families, particularly within the Iranian population. Through this exploration, we aspire to enhance our knowledge of the genetic factors influencing HL, paving the way for improved diagnostic strategies and potential interventions in similar cases.

Isolation of genomic DNA from blood leukocyte samples was performed using an established salting-out procedure. The concentration of DNA samples was assessed using a NanoDrop 1000 spectrophotometer. Subsequently, the isolated DNA was either stored at -20ºC for future use or subjected to immediate amplification.

Subsequently, DNA served as the instrumental medium for the creation of libraries and the implementation of targeted sequencing in our research endeavors. A bespoke Human capture array was meticulously designed to selectively capture the complete coding regions and intron/exon boundaries of

the specified genes linked to the pathogenesis of NSHL. The sequencing procedures were executed by the utilization of the WES method (Macrogen, Seoul, South Korea).

Alignment of sequence reads was conducted using the reference human genome build hg19 from UCSC, and subsequent annotation was performed utilizing datasets and tools. To refine the analysis, previously identified common variants (frequency >1%) and synonymous substitutions were filtered out, leveraging public databases such as the 1000 Genome Project (https://www.internationalgenome.org/), dbSNP (https://www.ncbi.nlm.nih.gov/snp/), and the gnomAD browser (https://gnomad.broadinstitute.org/).

To verify the accuracy of the novel variant identified by WES, Sanger sequencing was conducted on the patient and other family members. The PCR products underwent direct sequencing using the automated genetic analyzer (ABI 3100; Applied Biosystems). Subsequently, Sanger sequencing was conducted to confirm the segregation of the candidate variant within the family.

The analysis of DNA sequences within genes implicated in NSHL pathogenesis revealed a novel mutation in the *MYO15A* gene, which co-segregated among healthy family members (Figure 2). This mutation, identified as a novel deletion (c.1178_1179delAC) in exon 2 (NM_016239.4) of the *MYO15A* gene, induces a frameshift mutation. Specifically, it results in the substitution of Tyrosine to Serine at codon 393, potentially leading to a truncated MYO15A protein (p.Tyr393Serfs*38).

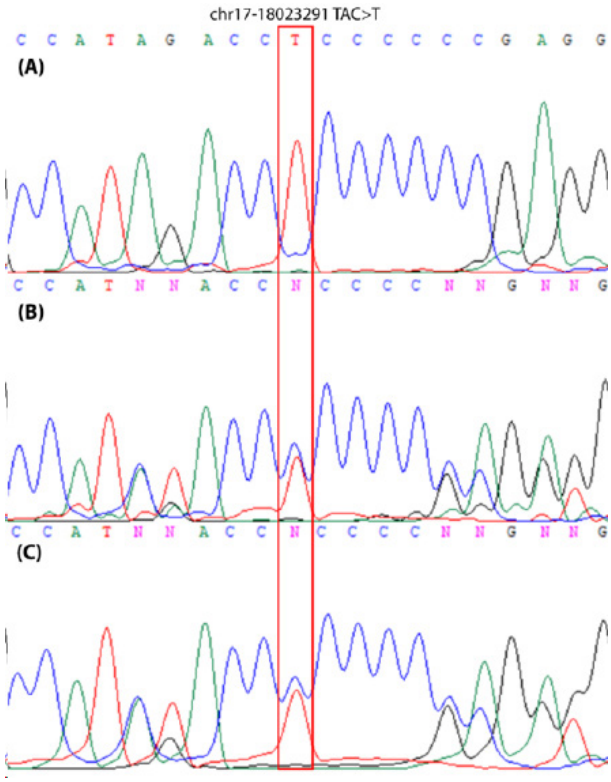

Fig. 2. *The genetic analysis results for the proband and his parents are depicted. Sanger sequencing for the proband (A) unveiled a novel homozygous c.1178_1179delAC mutation in exon 2 of the MYO15A gene. Notably, the identified mutation is found in a heterozygous state in his parents (B, C).*

This novel c.1178_1179delAC; p.Tyr393Serfs*38 deletion mutation in the *MYO15A* gene holds significant implications for ARNSHL. Thus, The deletion-induced frameshift disrupts the protein's structural integrity, potentially causing the loss of critical domains necessary for its proper function, and the dysfunctional MYO15A protein is unable to carry out its essential role in the development and maintenance of hair cells within the inner ear. This impairment leads to compromised auditory function, contributing to ARNSHL. Moreover, due to the premature termination, a truncated and likely non-functional MYO15A protein is produced.

Discussion

Our investigation into an Iranian family grappling with deafness has yielded a pivotal discovery by WES: the identification of a novel mutation in the *MYO15A* gene as the primary cause of the observed impairment within the family. This finding significantly advances our understanding of the genetic basis of deafness, especially within the Iranian population. The *MYO15A* gene's association with auditory function is well-established, and our study underscores its significance in familial cases of deafness. The detection of a novel mutation within this gene not only contributes to the expanding spectrum of genetic variants linked to hearing impairment but also highlights the intricate genetic diversity that may underpin such conditions, particularly within distinct ethnic groups like the Iranian population.

MYO15A's intricate structure with 66 exons and its coding protein, myosin XVa, play a pivotal role in the formation of stereocilia within the cochlea's hair cells.[11] Myosin XVa within the organ of Corti is specifically concentrated at the extremities of stereocilia. It operates as an actin-activated ATPase, utilizing ATP hydrolysis to traverse along actin filaments. The apex of a stereocilium is suggested as a potential location for mechano-electrical transduction, as well as the site for stereocilia expansion.[12] The proper functioning and formation of the mechanotransduction machinery depend on the presence of Myosin XVa. Myosins typically consist of one or two heavy chains along with several light chains. The tails of myosins are believed to bind to membranous compartments, allowing them to be displaced in relation to actin filaments.[13]

In a study by Xia H. et al.,[14] the authors presented compelling evidence of a causative frameshift mutation in the *MYO15A* gene within a Chinese family experiencing NSHL. This finding underscores the genetic complexity underlying NSHL and contributes to our understanding of the role of *MYO15A* mutations in the manifestation of this auditory disorder within specific populations. Moreover, earlier investigations have scrutinized families with NSHL to assess pathogenic genomic defects. These studies identified forty-three mutations in the *MYO15A* gene and determined that these modifications were responsible for ARNSHL.[15–17] Subsequently, Zhang F. et al.[18] reported three pathogenic mutations in the *MYO15A* gene c.3971C>A; p.A1324D, c.4011insA; p.Q1337Qfs*22, and c.9690+1G>A within a Chinese family affected by ARNSHL. Consistent with these discoveries, Asgharzade S. et al.[19] conducted an

assessment of mutations associated with NSHL within the Arab population in Southwest Iran, employing WES. Their study revealed a novel homozygous mutation, c.1047C>A; p.Y349*, in one of the twenty-five families. This mutation resulted in a premature stop codon, further emphasizing the genetic diversity underlying NSHL in this specific geographic and ethnic context. The identification of such novel mutations adds to the understanding of the genetic landscape of NSHL and highlights the importance of population-specific genetic studies in unraveling the intricacies of hereditary hearing disorders. These findings contribute valuable insights that may have implications for genetic counseling and diagnostic approaches in the affected population.[19]

This is the first report of c.1178_1179delAC mutation of the *MYO15A* gene in a patient affected by NSHL. The following evidence proves that this mutation can lead to NSHL: 1) WES exclusively identified this variant as the underlying cause of NSHL in the proband. 2) As depicted in Figure 2, Sanger sequencing validated the variant in both the patient and the unaffected family members, and, based on a recognized heterozygous mutation in the parents, indicates an AR inheritance pattern for *MYO15A*. 3) Furthermore, variant chr17-18023291 TAC>T located in exon 2 of the *MYO15A* gene within the ferm domain and tail region of the Myosin protein has been identified. This variant induces a novel reading frame (p.Tyr393Serfs*38), triggering premature encounter with a stop codon. Consequently, this event results in the synthesis of a truncated protein, which significantly disrupts the normal functioning of the protein, leading to impaired auditory function and contributing to the development of ARNSHL.

Our findings emphasize the substantial utility of employing WES in the context of consanguineous parents. This investigative approach proves to be a valuable and effective tool for uncovering both potential and novel mutations associated with ARNSHL. The comprehensive coverage of the exome allows for the detection of genetic variations that might otherwise go unnoticed, providing crucial insights into the genetic basis of ARNSHL in consanguineous family structures. This underscores the importance of adopting advanced genetic techniques, such as WES, to enhance our understanding of hereditary HL and contribute to the identification of causative mutations in affected individuals.

Conclusion

This study marks a significant milestone as it represents the first confirmed case of HL in an Iranian family by comprehensive genetic analysis, revealing a novel c.1178_1179delAC; p.Tyr393Serfs*38 mutation in the *MYO15A* gene. The identification of this unique mutation underscores the importance of genetic investigations in elucidating the diverse molecular underpinnings of hereditary hearing disorders within specific populations.

Competing Interests

The authors declare that they have no competing interests.

Ethical Considerations

All procedures performed in this study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments. Written informed consent was obtained from the family members for this publication.

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