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A NOVEL *LOXHD1* PATHOGENIC VARIANT IN GREECE: CASE STUDY OF A DNFB77 NONSYNDROMIC HEARING IMPAIRMENT

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Contributions:
A Study design/planning
B Data collection/entry
C Data analysis/statistics
D Data interpretation
E Preparation of manuscript
F Literature analysis/search
G Funds collection

Abstract

Introduction: Genetic deficits cause 80% of congenital prelingual hearing loss. Some 80% of these cases are nonsyndromic and most follow an autosomal recessive pattern of inheritance. More than 80 genes have so far been identified as pathogenic. In this study, we focus on a recently mapped gene, *LOXHD1*, whose mutated form causes DFNB77 hearing loss, and find a new variant.

Case report: This is a case study of a 7-year-old male who was admitted to our audiology department with progressive hearing impairment. Pure tone audiometry showed bilateral symmetric hearing impairment, especially at mid to high frequencies. Acoustic immittance tests were normal, but transient evoked otoacoustic emissions and auditory brainstem responses were abnormal for both ears. After physical, clinical, and imaging evaluations, the patient and his parents were genetically tested. Genetic screening showed *LOXHD1* hearing loss with a novel combination of alleles, the first such case diagnosed in Greece. The patient now has hearing aids and is being regularly monitored.

Conclusions: Autosomal recessive nonsyndromic hearing loss (ARNSHL), including DFNB77, is the most common sensory deficit in children. Our work describes the first *LOXHD1* hearing loss case diagnosed in Greece and adds a novel pathogenic variant to the list of pathogenic *LOXHD1* gene variants.

Key words: nonsyndromic hearing loss • genetic hearing loss • sensorineural pediatric hearing loss • *LOXHD1* • DFNB77

NOWY PATOGENICZNY WARIANT GENU *LOXHD1* W GRECJI: STUDIUM PRZYPADKU IZOLOWANEGO NIEDOSŁUCHU ZWIĄZANEGO Z MUTACJĄ DNFB77

Streszczenie

Wprowadzenie: Uwarunkowania genetyczne są przyczyną 80% przypadków wrodzonego niedosłuchu prelingwalnego. Około 80% spośród tych przypadków stanowią niedosłuchy izolowane, większość jest dziedziczona autosomalnie recesywnie. Ponad 80 genów zostało do tej pory zidentyfikowanych jako patogeniczne. W przedstawionym badaniu opisujemy niedawno zmapowany gen *LOXHD1*, którego zmutowana forma powoduje niedosłuch DFNB77 i zidentyfikowaliśmy nowy wariant.

Studium przypadku: Prezentujemy przypadek 7-letniego chłopca przyjętego w naszej klinice audiologii z powodu postępującego niedosłuchu. Audiometria tonalna wykazała obustronny symetryczny niedosłuch, szczególnie w zakresie od średnich do wysokich częstotliwości. Wynik audiometrii impedancyjnej był w normie, ale wyniki badania otoemisji akustycznych wywołanych trzaskiem i odpowiedzi słuchowych pnia mózgu były nieprawidłowe dla obojga uszu. Wykonano badania fizykalne, kliniczne i obrazowe, a następnie pacjent i jego rodzice przeszli testy genetyczne. Przesiewowe badanie genetyczne wykazało niedosłuch *LOXHD1* z nową kombinacją alleli – pierwszy taki przypadek zdiagnozowany w Grecji. Obecnie pacjent nosi aparaty słuchowe i podlega regularnej kontroli.

Wnioski: Izolowany niedosłuch autosomalnie recesywny (ARNSHL), w tym DFNB77, to najczęściej występujący rodzaj deficytu czuciowego u dzieci. Nasz artykuł opisuje pierwszy przypadek niedosłuchu *LOXHD1* zdiagnozowany w Grecji i dodaje nowy patogeniczny wariant do listy patogenicznych wariantów genu *LOXHD1*.

Słowa kluczowe: niedosłuch izolowany • niedosłuch uwarunkowany genetycznie • niedosłuch odbiorczy u dzieci • *LOXHD1* • DFNB77

Introduction

Hearing loss in children is common and affects nearly 1 of 5 children in the United States by the age of 18 [1]. Of every 1000 newborns, about 1–3 will receive a diagnosis of permanent hearing impairment [2]. Congenital hearing loss is predominantly due to genetic causes whereas acquired cases are in the minority [3]. Hearing loss and deafness can be characterised as syndromic or nonsyndromic [4]. In syndromic cases, hearing impairment is a symptom accompanying systemic disorders or malformations of the external ears or other organs [5]. In nonsyndromic cases, defects of the middle and/or inner ear can be present, although external ear deformities or systematic abnormalities are absent. A further classification categorises hearing loss as prelingual, when the impairment precedes language development, and postlingual when the hearing disorder follows language establishment [6]. There is a mnemonic 80% rule: genetic deficits cause 80% of congenital prelingual hearing loss, 80% of those cases are nonsyndromic, and 80% of nonsyndromic cases follow an autosomal recessive pattern of inheritance.

The hereditary pattern of nonsyndromic hearing impairment is genetically heterogeneous because thousands of causative allele variants have been reported in more than 110 genes [4]. More than 80 genes have been identified as the genetic basis of autosomal recessive hearing loss [7], with pathogenic variants in the *GJB2* gene contributing over half the cases [8,9]. In the present study, we focused on a recently mapped gene, *LOXHD1*, the mutated form of which causes DFNB77 progressive hearing loss.

With the development of genetic testing technology, rarer cases will enter the literature. In this work, we present the first *LOXHD1* hearing loss case diagnosed in Greece together with a literature review on *LOXHD1*-related hearing impairments.

Material and methods

Family report

All procedures were approved by the Ethics Review Committee of Agia Sofia Children Hospital, Athens, Greece. Written informed consent was obtained from the parents of the patient involved. A 7-year-old boy was admitted to our audiology department, with a report of progressively deteriorating hearing. After history taking, physical examination, and audiological testing, a moderately severe sensorineural hearing loss was diagnosed. A three-generation family history was taken, and a family audioprofile was compiled. Further testing, including cardiac, renal, and ophthalmologic, was ordered. To check for possible anatomical variations, MRI imaging of the temporal bone was performed. The clinical findings, the negative family history, and the progressive character of hearing loss indicated nonsyndromic hearing loss.

Genetic analysis

Molecular genetic testing was done on both the patient and his parents. To look for pathogenic variants, we used the Clinical Exome Solution v2 kit (SOPHiA Genetics, Switzerland). Targeted next-generation sequencing (NGS) was performed to screen for gene mutations related to hereditary hearing loss in the patient's genome. The MiSeq System (Illumina, San Diego, CA) was used, a platform which detects mutations in the coding regions ±5 bp of intronic regions of 4,493 genes, the entire mitochondrial genome, and ~200 non-coding variants with known pathogenicity in deep introns/enhancer/promoter genes. The abovementioned NGS technique detected several polymorphisms in the clinical exome kit, which are generally not noted because they are clinically irrelevant. Data filtering was performed using the Illumina MiSeq Reporter and Variant Studio data analysis software (Illumina, San Diego, CA). Bioinformatic analysis was performed using the SOPHiA DDM platform (SOPHiA Genetics, Switzerland). Sanger sequencing and MLPA (MRC The Netherlands) on specific exons validated any pathogenic variants in the clinical exome kit. Detection of copy number variation (CNV) in genes associated with hearing loss, although useful in genetic diagnosis, was not performed. All testing was done according to the manufacturer's instructions.

Results

At the first examination, we detected moderately severe sensorineural hearing loss with no other symptoms. Pure tone audiometry showed bilateral symmetric hearing impairment, especially at mid to high frequencies. Acoustic immittance tests were normal, but transient evoked otoacoustic emissions and auditory brainstem responses were abnormal in both ears. There were no signs of tinnitus, dizziness, or other vestibular dysfunction. There was no history of ear infection or any external ear malformation. The symptoms of hearing loss were established a few months ago. Medical and family history were free of health problems associated with hearing impairment. During gestation, ototoxic medications were not used and there were no viral infections.

Following our diagnosis of hearing loss, we suggested imaging of the temporal bone, and a thin slice MRI excluded the possibility of inner ear malformations such as Mondini deformity, Michel aplasia, or enlarged vestibular aqueduct. After eliminating any anatomical or systemic deficits, we were led towards a molecular diagnosis of congenital hearing loss.

Targeted next-generation sequencing was performed, and this revealed two rare mutations in the *LOXHD1* gene (NM_144612.6). The NM_144612.6: c.3169C>T (p.Arg1057*) in exon 20 and NM_144612.6: c.4480C>T (p.Arg1494*) in exon 29 were detected in heterozygosity. Sanger sequencing on exons 20 and 29 verified the results, and confirmed a case of ARNSHL (autosomal recessive nonsyndromic hearing loss). Evaluation of the parents with PCR and Sanger sequencing on the same exons disclosed the origin of the mutated variants. A c.3169C>T (p.Arg1057*) mutation was detected in the patient's father, while the mother had a mutation of c.4480C>T (p.Arg1494*).

Over the following 3 months, the patient was under close follow-up to document the progress of hearing loss. Although most *LOXHD1* cases (62%) are associated with

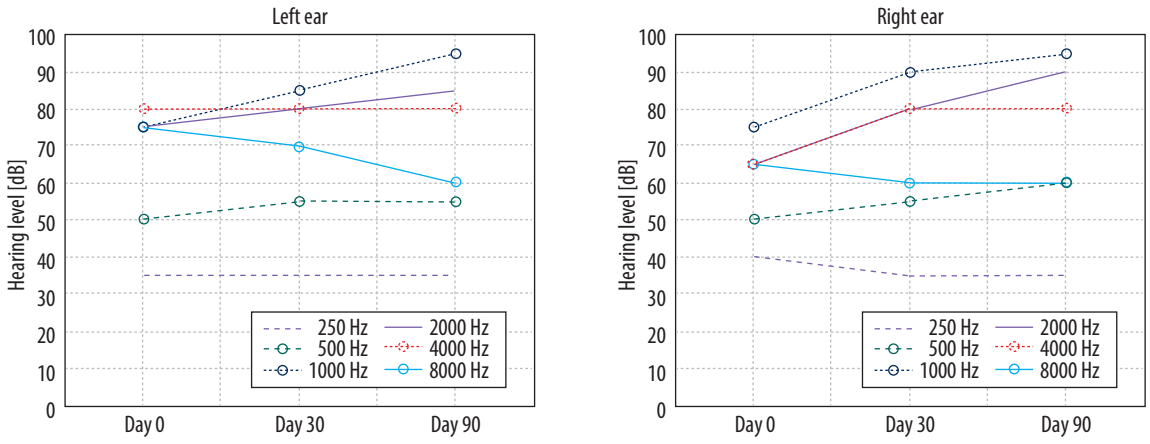

Figure 1. Audiograms for the right and left ears during the follow-up period

Table 1. *LOXHD1* gene variations and their clinical features (NM_144612.6 and NP_653213.6)

Nucleotide change (protein change)	HL onset	Severity of HL	Progression	Population
c.3169C>T (p.Arg1057*) c.4480C>T (p.Arg1494*)	childhood (7 y.o.)	moderately severe	progressive	Greek
c.3169C>T (p.Arg1057*) c.6353G>A (p.Gly2118Glu)	congenital	severe	stable	Dutch Wesdorp et al. [18]
c.4480C>T (p.Arg1494*) c.4480C>T (p.Arg1494*)	NA	NA	NA	Turkey Diaz-Horta et al. [19]
c.4480C>T (p.Arg1494*) c.4480C>T (p.Arg1494*)	congenital	mild–moderate	NA	American Lebeko et al. [20]
c.4480C>T (p.Arg1494*) c.4526G>A (p.Gly1509Glu)	40 y.o.	severe–profound	progressive	American Eppsteiner et al. [16]
c.4480C>T (p.Arg1494*) c.5869G>T (p.Glu1957*)	childhood (1–6 y.o.)	moderate–severe	stable	Japanese Mori et al. [22]
c.4480C>T (p.Arg1494*) c.6598delG (p.Asp2200Metfs*22)	childhood	severe–profound	NA	American Lebeko et al. [20]

a down-sloping audiogram with mild-to-moderate hearing loss at low frequencies [10], our patient's hearing deteriorated mainly at mid frequencies of 1–2 kHz (**Figure 1**).

Hearing aids were then prescribed, and the patient remains on regular surveillance. The patient and his family declined other treatment options such as a cochlear implant.

Discussion

The *LOXHD1* gene is located on chromosome 18q12-q21 and contains 2,211 amino acids in its 43 exons. *LOXHD1* is expressed along the plasma membrane of the hair cell stereocilia. Proteins encoded by *LOXHD1* are unique and consist entirely of 15 PLAT (polycystin/lipoxygenase/α-toxin) domains [11] which are involved in the targeting of proteins to the plasma membrane [12]. PLAT domains interact with lipids and proteins to form a β-sandwich domain [13]. In a mutated mouse model (samba mouse), *LOXHD1* is found at low levels on postnatal day two, but increases at postnatal day 10. Although stereocilia development is unaffected, hair cell function is disturbed, and the hair cells eventually degenerate [14]. Mutations of *LOXHD1*-PLAT10 drastically restrict inner hair cell mechanotransduction shortly after birth. In this way, hearing loss occurs, even though the hair bundle largely maintains its structure.

LOXHD1 is mainly expressed in the stereocilia of cochlear hair cells, and less intensively in vestibular hair cells [15]. Eppsteiner et al. [16] showed that this selective expression in cochlear stereocilia leads to better outcomes in patients with *LOXHD1* variation who receive a cochlear implant. In addition to stereocilia, cochlear and vestibular hair cells contain a single kinocilium, although it is lost in the cochlea as the hair cells mature at the same time as *LOXHD1* expression increases. This suggests that the protein might operate as a stabiliser for the stereociliary bundle following kinocilium degeneration.

In a Japanese study the prevalence of *LOXHD1* hearing loss was found to be 0.37% (28/8074) of sensorineural hearing loss (SNHL) probands. Data from studies in the USA, Netherlands, and Italy estimated the incidence of pathogenic variants in *LOXHD1* gene to be 0.71% (8/1119), 1.5% (3/200), and 0.97% (1/103), respectively [17].

Despite the rarity of *LOXHD1* mutations, our work uncovered a unique combination of alleles, an NM_144612.6: c.3169C>T (p.Arg1057*) and NM_144612.6: c.4480C>T (p.Arg1494*) heterozygous variant. Interestingly, previous work has detected different combinations of the two mutated alleles (**Table 1**).

Most reports conclude that hearing loss due to *LOXHD1* is a congenital disorder or at least of early onset, although some have seen a more delayed onset of hearing loss, from 7 to 40 years of age [16]. A wide range is also found in the severity and progressiveness of hearing loss, fluctuating from moderate to profound at mid and high frequencies, with audiological stabilisation over time. However, there are cases of gradual deterioration [21]. Although phenotypic diversity characterises *LOXHD1* mutations, Wesdorp et al. [18] found that the severity and progression of hearing loss could not be clearly associated with the type (nonsense or missense) or location of the mutated variant. Environmental factors and genetic modifiers may explain these differences. In the vast majority of DFNB77 patients, the genetic analysis leads to nonsense mutations [23], which are predicted to insert a premature stop codon and cause progressive ARNSHL. The *LOXHD1* gene is expressed in hair cells and is associated with progressive ARNSHL, suggesting it may be caused by an age-dependent hair cell failure mechanism. Interestingly, recessive inheritance is linked with non-progressive hearing loss, whereas dominant hearing loss inheritance typically leads to postlingual progressive hearing loss [24].

As was apparent in our study, normal vestibular function is a characteristic of *LOXHD1* hearing loss. Worldwide, no other sensorineural or automotor deficit has been found in any such patients, although Riazuddin et al. [25] and Stehouwer et al. [26] found a pathogenic association between heterozygous missense variants in *LOXHD1* and late-onset Fuchs corneal dystrophy (FCD). Before *LOXHD1*, *SLC4A11* had also been associated with this dominantly inherited corneal genetic disorder [27,28]. Our patient and his relatives (parents and grandparents) who had normal audiometric tests did not show corneal dysfunction. Similar findings apply to most of the literature, suggesting that heterozygous pathogenic variants in *LOXHD1* might be better considered as a risk factor for FCD.

Conclusions

ARNSHL, including DFNB77, is the most common sensory deficit in children. Genetic testing is vital, especially in nonsyndromic cases like *LOXHD1* HL where the lack of a specific phenotypic profile hinders immediate and effective diagnosis. Our study presents a novel combination of alleles and is notable as being the first DNFB77 nonsyndromic hearing impairment case diagnosed in Greece.

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