J Hear Sci, 2021; 11(4): 72–76
DOI: 10.17430/JHS.2021.11.4.8

ISSN: 2083-389X,
eISSN: 2084-3127

POSTLINGUAL SENSORINEURAL HEARING LOSS DUE TO A KNOWN *TBC1D24* GENE ALTERATION

Dominika Oziębło[1,2,A-F], Marcin L. Leja[1,2,B-E], Natalia Bałdyga[1,B-C,E], Anna Sarosiak[1,2,B-C,E], Henryk Skarzynski[3,B,E,G], Monika Ołdak[1,A-G]

[1] Department of Genetics, Institute of Physiology and Pathology of Hearing, Poland
[2] Postgraduate School of Molecular Medicine, Medical University of Warsaw, Poland
[3] Oto-Rhino-Laryngology Surgery Clinic, Institute of Physiology and Pathology of Hearing, Poland

Corresponding author: Monika Ołdak; Department of Genetics, Institute of Physiology and Pathology of Hearing, Mochnackiego 10, 02-042, Warsaw, Poland; email: m.oldak@ifps.org.pl

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

Background: Genetically determined hearing loss (HL) can be inherited in two major ways - autosomal recessive or dominant. Autosomal dominant hearing loss (ADHL) is usually postlingual and progressive. To date, 50 different genes have been demonstrated to be causally involved in its development. In Polish ADHL patients, *TBC1D24* pathogenic variants are a frequent cause of the disease.

Case report: A three-generation Polish family with ADHL was recruited for the study. An audiological assessment was conducted and next-generation sequencing of a custom HL multigene panel (n = 237 genes) was performed in the index patient. The presence and segregation of the detected variant with HL was verified by Sanger sequencing. All affected individuals had postlingual progressive HL, mainly affecting high frequencies. In this study, a very rare previously reported p.Ser178Leu variant in *TBC1D24* was identified in all family members with HL.

Conclusions: Our study provides an independent confirmation of the pathogenic role of *TBC1D24* p.Ser178Leu in HL. In individuals with ADHL-related *TBC1D24* pathogenic variants, the cochlear component of the auditory system is affected. Patients may also report tinnitus but usually do not complain of vertigo.

Keywords: *TBC1D24* • autosomal dominant hearing loss (ADHL) • next generation sequencing • progressive hearing loss

POSTLINGWALNY NIEDOSŁUCH ODBIORCZY SPOWODOWANY ZNANYM USZKODZENIEM GENU *TBC1D24*

Streszczenie

Wprowadzenie: Główne formy dziedziczenia niedosłuchu uwarunkowanego genetycznie to autosomalny recesywny lub dominujący. Niedosłuch autosomalny dominujący (ADHL) jest zwykle postlingwalny i postępujący. Do tej pory znanych jest 50 różnych genów powiązanych przyczynowo z rozwojem takiego niedosłuchu. Wśród polskich pacjentów z ADHL częstą przyczyną choroby są patogenne warianty genu *TBC1D24*.

Opis przypadku: W badaniu wzięła udział trzypokoleniowa polska rodzina z ADHL. U probanda przeprowadzono ocenę audiologiczną oraz sekwencjonowanie następnej generacji z wykorzystaniem autorskiego panelu wielogenowego ukierunkowanego na niedosłuch (n = 237 genów). Występowanie i segregacja wykrytego wariantu z niedosłuchem zostały potwierdzone sekwencjonowaniem metodą Sangera. Wszystkie chore osoby miały postępujący niedosłuch postlingwalny, przeważnie w zakresie wysokich częstotliwości. W naszym badaniu u wszystkich członków rodziny, którzy mieli niedosłuch, zidentyfikowano bardzo rzadko występujący, opisany wcześniej wariant p.Ser178Leu genu *TBC1D24*.

Wnioski: Niniejsze badanie stanowi niezależne potwierdzenie patogennej roli wariantu p.Ser178Leu w genie *TBC1D24* w powstawaniu niedosłuchu. U osób z patogennymi wariantami *TBC1D24* odpowiedzialnymi za ADHL upośledzona jest funkcja ślimaka w narządzie słuchu i równowagi. Pacjenci mogą także zgłaszać występowanie szumów usznych, ale zwykle nie skarżą się na zawroty głowy.

Słowa kluczowe: *TBC1D24* • niedosłuch autosomalny dominujący (ADHL) • sekwencjonowanie następnej generacji • niedosłuch postępujący

Introduction

Hearing loss (HL) is one of the most common sensory disorders in developed countries, and it has been estimated to affect almost 460 million people worldwide. Many different environmental factors (e.g. severe prematurity, hyperbilirubinemia, infection, ototoxic drugs or noise) may cause HL, but the genetic background also plays an important role in its development [1]. The most frequent type of genetically determined HL is autosomal recessive (ARHL), with a significant contribution to pathogenic variants located in the DFNB1 *locus*. Patients with genetic alterations affecting the DFNB1 *locus* mainly have profound prelingual HL, and it accounts for approx. 20% of all congenital HL cases. The prevalence of HL increases with age, and in individuals with progressive postlingual HL and a positive family history, autosomal dominant inheritance is usually recognized [2].

Autosomal dominant hearing loss (ADHL) is the second most common type of HL. It is caused by a heterozygous pathogenic variant that disrupts proper functioning of the

involved protein. Families with ADHL usually have a characteristic pedigree with a vertical transmission of HL, which can be inherited either from the mother or father. For a patient with ADHL, the risk of having a child with the same type of HL is 50%. To date, 64 loci with 50 different genes have been causally involved in the development of ADHL (https://hereditaryhearingloss.org; accessed 03/2021).

The *TBC1D24* gene (OMIM *613577) has several different isoforms expressed in multiple human tissues. The highest expression level of *TBC1D24* gene has been observed in the brain, but there are also data reporting *TBC1D24* transcripts in testis, skeletal muscle, heart, kidney, lung, and liver [3]. Expression of mouse *Tbc1d24* gene has also been reported in the cochlea, i.e., stereocilia of inner and outer hair cells and spiral ganglion. The *TBC1D24* gene encodes a member of Tre2-Bub2-Cdc16 (TBC) domain-containing RAB-specific GTPase-activating proteins. It consists of two main domains, i.e., the TBC domain, which coordinates Rab proteins and other GTPases for the proper transport of intracellular vesicles [4,5], and the TLDc domain, which is probably involved in oxidative stress response [6].

In this study, we have searched for the genetic cause of HL in a three-generation Polish family. We have applied a high throughput sequencing method and identified a *TBC1D24* pathogenic variant.

Material and methods

Patients and clinical diagnosis

A three-generation Polish family with multiple individuals with HL was recruited for the study at the Institute of Physiology and Pathology of Hearing (Figure 1A). All tested subjects gave informed consent for participation in the study, in accordance with the tenets of the Declaration of Helsinki. The study was approved by the local ethics committee (KB.IFPS.25/2017).

Assessment of auditory function in the index patient (III.3) was performed with pure-tone audiometry (PTA). Hearing thresholds for air and bone conduction were determined at frequencies 125–8000 Hz and 500–4000 Hz respectively with an AC40 clinical audiometer (Interacoustics, Middelfart, Denmark) using the 10/5 dB descending–ascending threshold estimation procedure [7]. The medical history and previous audiological data involving impedance audiometry, otoacoustic emissions, and ABRs were also analyzed.

Targeted next-generation sequencing

Genomic DNA was isolated from whole blood samples with a standard salting out procedure and from buccal swab samples using a Maxwell RSC Buccal Swab DNA Kit and Maxwell RSC Instrument (Promega, Walldorf, Germany). The quality and quantity of the isolated DNA were assessed using agarose gel electrophoresis and a Qubit HS Assay Kit with Qubit 2.0 fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). In the index patient (III.3), a custom multigene panel (Roche, Basel, Switzerland) containing 237 HL genes was performed according to the manufacturer's protocol, and libraries were run on a MiSeq using 2 × 75 bp paired-end reads. Bioinformatic analyses were performed using a previously described pipeline [8]. Annotated variants were converted to the MS Access format for further expert analyses. An Integrative Genomics Viewer was used to visualize candidate variants [9].

Genetic variants identified by high throughput sequencing were further analyzed based on (i) their population frequencies in the Genome Aggregation Database (gnomAD) (https://gnomad.broadinstitute.org), the UK10K Project (https://www.uk10k.org) and the NHLBI GO Exome Sequencing Project (ESP) (https://evs.gs.washington.edu/EVS) (all accessed 03/2021) as well as (ii) computational pathogenicity predictions generated with the SIFT [10], PolyPhen-2 [11], MutationTaster2 [12], LRT [13], CADD [14], REVEL [15], and SpliceAI [16] algorithms. Evolutionary conservation was evaluated using the GERP++ score [17]. Multiple protein sequence alignment was performed using COBALT [18] and variant localization across evolutionary diverse species was visualized with the Jalview v2.10.5 software [19]. Analyzed sequences included TBC1D24 orthologues from human (*Homo sapiens*) [NP_001186036.1], rhesus macaque (*Macaca mulatta*) [NP_001244494.1], house mouse (*Mus musculus*) [NP_001157319.1], brown rat (*Rattus norvegicus*) [NP_001099239.1], cattle (*Bos taurus*) [NP_001039761.1], red junglefowl (*Gallus gallus*) [NP_001244200.1], western clawed frog (*Xenopus tropicalis*) [NP_001072701.1] as well as zebrafish (*Danio rerio*) [XP_021326084.1].

Sanger sequencing

Confirmation of the presence of the candidate pathogenic variant and family segregation analyses were performed using Sanger sequencing. Exon 2 of the *TBC1D24* gene was amplified with a forward 5'-GATGAAACGGGTTGT GGCTCT and reverse 5'-CAGACCGTTGACCCTCCATAG primers. Next, PCR products were labeled with the BigDye Terminator cycle sequencing kit v3.1 (Applied Biosystems, Foster City, CA, USA), sequenced with a 3500xL Genetic Analyzer (Applied Biosystems), and analyzed using Variant Reporter software v1.1 (Applied Biosystems).

Results

The index patient suffered from postlingual HL from the age of 30. It was diagnosed 5 years later and pure tone audiometry performed at the age of 39 revealed bilateral mild to moderate HL affecting mainly high frequencies (Figure 1B). Additional audiological diagnostics confirmed cochlear involvement (absence of otoacoustic emissions and normal ABR results). The thresholds of stapedial muscle reflexes were increased. During subsequent appointments, chronic tinnitus but no vertigo was reported. The patient was fitted with hearing aids at the age of 44 with a poor effect.

High throughput genetic testing performed on the proband's DNA sample generated a list of 5773 genetic variants that met quality criteria. After filtering the data, the c.533C>T genetic variant in the *TBC1D24* gene was selected for further family segregation studies (Figure 1AC). The majority of computational algorithms predicted the damaging role of the c.533C>T transition. This variant results in serine-to-leucine amino acid substitution at the protein position 178 (p.Ser178Leu). Conservation analyses showed 100% identity of the analyzed region among all tested species (Figure

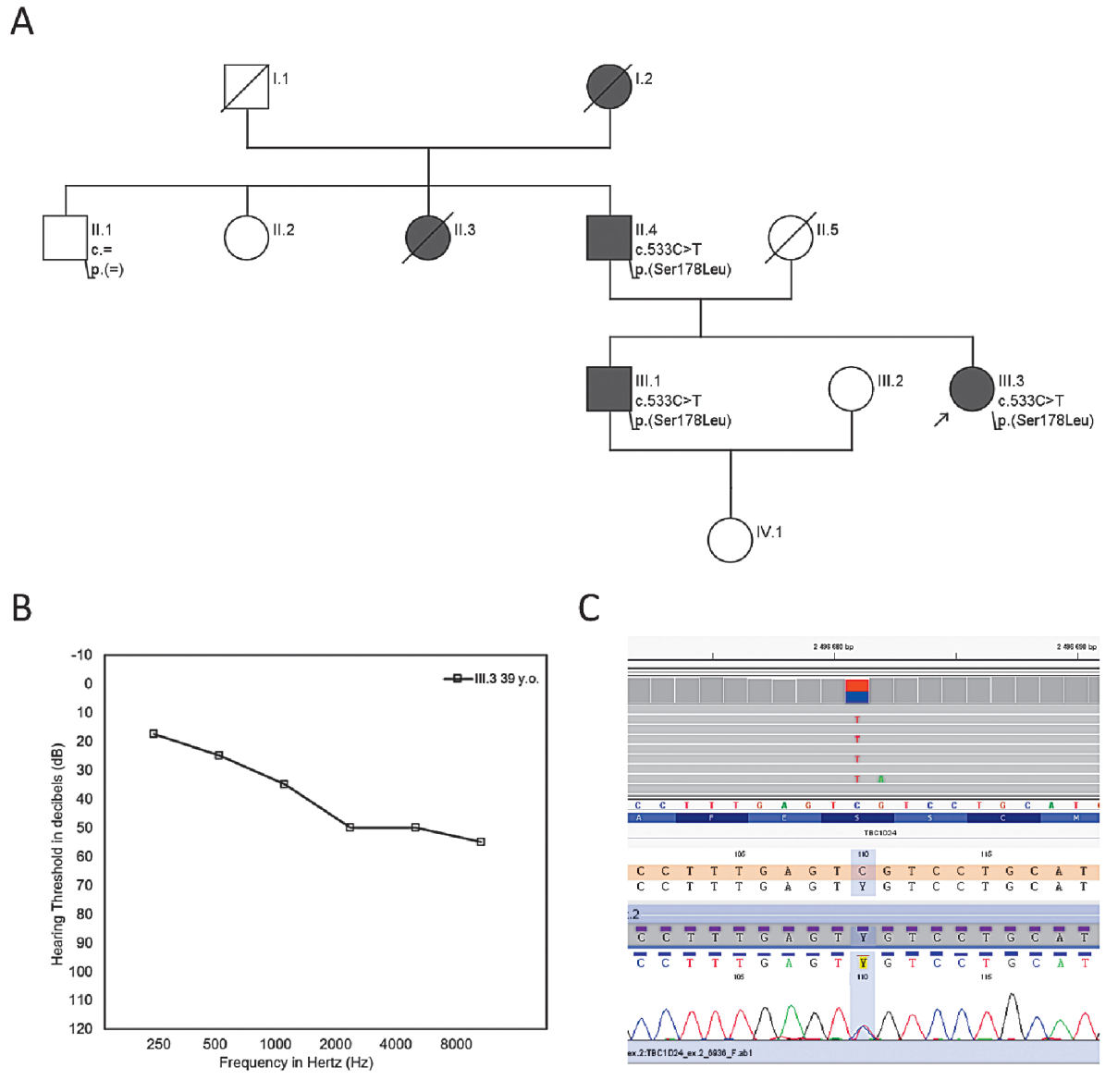

Figure 1. Results of genetic and audiological assessment in a Polish family with autosomal dominant HL. **A)** Family pedigree. The proband is marked with an arrow. Affected patients are indicated by black symbols and unaffected individuals are indicated by open symbols. Diagonal lines denote deceased individuals. **B)** Audiogram of index patient (III.3) showing mainly high frequency HL. **C)** NGS results visualized with Integrative Genomic Viewer software and corresponding chromatogram showing a heterozygous cytosine-to-thymine transition detected by Sanger sequencing. **D)** Multiple protein sequence alignment of human TBC1D24 protein in the region encompassing the p.Ser178Leu pathogenic variant.

1D) with a GERP++ score of 5.43, which confirmed its high conservation. The identified variant is very rare and present in the gnomAD population database with an allele frequency of only 0.000008. For the first time p.Ser178Leu was linked with the development of HL in 2014 [20,21] and reported in the Human Gene Mutation Database (www.hgmd.cf.ac.uk/ac/index.php) with accession number CM146963. Based on the pathogenicity criteria mentioned above, this variant was classified as probably pathogenic. Family segregation analysis confirmed the presence of the p.Ser178Leu genetic variant in all subjects with HL (II.4 and III.1) but not in an unaffected family member (II.1).

Discussion

The introduction of high throughput genetic testing allowed us to identify a known *TBC1D24* p.Ser178Leu pathogenic variant in a Polish family with ADHL. In the literature, this variant was reported in 2014 in an American and a Chinese family as the first *TBC1D24* variant causative for ADHL [20,21]. This variant was also detected in another Polish family with ADHL [22], but it was not assessed whether both families have a common ancestor based on the available data. Altogether, ADHL-related *TBC1D24* variants have been reported in 9 families worldwide (including the present study). In this group, p.Ser178Leu was the most frequently found (4 families), while p.Asn307His was detected in 2 families of Austrian and British origin [23]. The remaining 3 families have three different *TBC1D24* variants, i.e., p.Asp185Asn, p.His487Gln, or p.His487Leu [22].

The involvement of particular genes in ADHL development varies depending on the studied population. Identified mutations localize mainly in *TECTA*, *KCNQ4*, *WFS1*, or *MYO6* genes, but the contribution of a single gene usually does not exceed 12% [24]. Our recent extensive genetic studies performed in 102 ADHL families revealed an unexpectedly high frequency (4%) of *TBC1D24* pathogenic variants, making this gene one of the most commonly involved in the development of ADHL, at least in Polish patients [22]. The high genetic heterogeneity of ADHL justifies the use of high throughput sequencing approaches that enable parallel testing of a number of selected genes with a high detection rate of causative variants [25].

Patients with *TBC1D24*-related HL have progressive sensorineural HL that arises due to impaired function of the TBC1D24 protein in the cochlea, but the mechanism of its action remains unknown. On average, the progression of HL in patients with *TBC1D24*-related HL is approximately 0.69 dB/year, although the progression rates for a particular variant can differ slightly. The majority of patients with *TBC1D24*-related ADHL do not require cochlear implantation, and their HL is usually accompanied by tinnitus, but they do not usually complain of vertigo [22].

Funding

This work was funded by the Institute of Physiology and Pathology of Hearing and Sonata BIS6 grant of the National Science Centre 2016/22/E/NZ5/00470.

References

1. Korver AM, Smith RJ, Van Camp G, Schleiss MR, Bitner-Glindzicz MA, Lustig LR et al. Congenital hearing loss. Nat Rev Dis Primers, 2017; 3: 16094.
2. Smith RJH, Shearer AE, Hildebrand MS. Deafness and Hereditary Hearing Loss Overview 1999 [updated 2017 July 27. Available from http://www.ncbi.nlm.nih.gov/books/NBK1434/.
3. Falace A, Filipello F, La Padula V, Vanni N, Madia F, De Pietri Tonelli D et al. TBC1D24, an ARF6-interacting protein, is mutated in familial infantile myoclonic epilepsy. Am J Hum Genet, 2010; 87(3): 365–70.
4. Campeau PM, Kasperaviciute D, Lu JT, Burrage LC, Kim C, Hori M et al. The genetic basis of DOORS syndrome: an exome-sequencing study. Lancet Neurol, 2014; 13(1): 44–58.
5. Sheehan P, Waites CL. Coordination of synaptic vesicle trafficking and turnover by the Rab35 signaling network. Small GTPases, 2019; 10(1): 54–63.
6. Finelli MJ, Oliver PL. TLDc proteins: new players in the oxidative stress response and neurological disease. Mamm Genome, 2017; 28(9-10): 395–406.
7. Recommended procedure for pure-tone bone-conduction audiometry without masking using a manually operated instrument [Technical note]. Br J Audiol, 1985; 19(4): 281–2.
8. Ozieblo D, Pazik J, Stepniak I, Skarzynski H, Oldak M. Two novel pathogenic variants confirm *RMND1* causative role in Perrault syndrome with renal involvement. Genes (Basel), 2020; 11(9): 1060.
9. Robinson JT, Thorvaldsdottir H, Winckler W, Guttman M, Lander ES, Getz G et al. Integrative genomics viewer. Nat Biotechnol, 2011; 29(1): 24–6.
10. Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nat Protoc, 2009; 4(7): 1073–81.
11. Adzhubei I, Jordan DM, Sunyaev SR. Predicting functional effect of human missense mutations using PolyPhen-2. Curr Protoc Hum Genet, 2013; Chapter 7: Unit 7 20.
12. Schwarz JM, Cooper DN, Schuelke M, Seelow D. MutationTaster2: mutation prediction for the deep-sequencing age. Nat Methods, 2014; 11(4): 361–2.
13. Chun S, Fay JC. Identification of deleterious mutations within three human genomes. Genome Res, 2009; 19(9): 1553–61.
14. Rentzsch P, Witten D, Cooper GM, Shendure J, Kircher M. CADD: predicting the deleteriousness of variants throughout the human genome. Nucleic Acids Res, 2019; 47(D1): D886–D894.
15. Ioannidis NM, Rothstein JH, Pejaver V, Middha S, McDonnell SK, Baheti S et al. REVEL: an ensemble method for predicting the pathogenicity of rare missense variants. Am J Hum Genet, 2016; 99(4): 877–85.
16. Jaganathan K, Kyriazopoulou Panagiotopoulou S, McRae JF, Darbandi SF, Knowles D, Li YI et al. Predicting splicing from primary sequence with deep learning. Cell, 2019; 176(3): 535–48.e24.
17. Davydov EV, Goode DL, Sirota M, Cooper GM, Sidow A, Batzoglou S. Identifying a high fraction of the human genome to be under selective constraint using GERP++. PLoS Comput Biol, 2010; 6(12): e1001025.
18. Papadopoulos JS, Agarwala R. COBALT: constraint-based alignment tool for multiple protein sequences. Bioinformatics, 2007; 23(9): 1073–9.
19. Waterhouse AM, Procter JB, Martin DM, Clamp M, Barton GJ. Jalview Version 2: a multiple sequence alignment editor and analysis workbench. Bioinformatics, 2009; 25(9): 1189–91.
20. Azaiez H, Booth KT, Bu F, Huygen P, Shibata SB, Shearer AE et al. *TBC1D24* mutation causes autosomal-dominant nonsyndromic hearing loss. Hum Mutat, 2014; 35(7): 819–23.

21. Zhang L, Hu L, Chai Y, Pang X, Yang T, Wu H. A dominant mutation in the stereocilia-expressing gene *TBC1D24* is a probable cause for nonsyndromic hearing impairment. Hum Mutat, 2014; 35(7): 814–8.
22. Ozieblo D, Leja ML, Lazniewski M, Sarosiak A, Tacikowska G, Kochanek K et al. *TBC1D24* emerges as an important contributor to progressive postlingual dominant hearing loss. Sci Rep, 2021; 11: 10300.
23. Parzefall T, Frohne A, Koenighofer M, Neesen J, Laccone F, Eckl-Dorna J et al. A novel variant in the *TBC1D24* lipid-binding pocket causes autosomal dominant hearing loss: evidence for a genotype–phenotype correlation. Front Cell Neurosci, 2020; 14: 585669.
24. Oza AM, DiStefano MT, Hemphill SE, Cushman BJ, Grant AR, Siegert RK et al. Expert specification of the ACMG/AMP variant interpretation guidelines for genetic hearing loss. Hum Mutat, 2018; 39(11): 1593–613.
25. Shearer AE, Smith RJ. Massively parallel sequencing for genetic diagnosis of hearing loss: the new standard of care. Otolaryngol Head Neck Surg, 2015; 153(2): 175–82.