Schwarz *et al.*
The Egyptian Journal of Otolaryngology (2024) 40:131
https://doi.org/10.1186/s43163-024-00661-x

The Egyptian Journal of Otolaryngology

CASE REPORT Open Access

A *TBC1D24* gene variant coincides with *STRC* compound heterozygosity in a family with hearing loss: a case report

Martin Schwarz[1,4], Kamila Hanková[3], Markéta Havlovicová[1], Radka Kremlíková Pourová[1*] and Jan Bouček[2]

Abstract

Background Hearing loss is a common inborn neurosensory condition. Hearing loss is very heterogeneous, and while screening programs exist for children, adolescents and adults with late-onset hearing loss often do not get referrals to geneticists.

Objective To diagnose the cause of hearing impairment in two related late-onset hearing loss patients—father and son—on a molecular level. Both underwent audiological examinations, and both had moderate hearing loss.

Case presentation.

We used massive parallel sequencing, Sanger sequencing, MLPA, and standard audiological methods. We identified an inherited autosomal dominant likely causative variant in the *TBC1D24* gene of both patients. They did not show any other *TBC1D24* spectrum-related symptoms. Furthermore, the younger patient was found to be compound heterozygous for two variants in *STRC* gene.

Conclusions Only a few dozen *TBC1D24* hearing loss patients have been reported. On the contrary, *STRC* is a common hearing loss cause. We speculate that in the younger patient, the phenotype is caused by a combination of effects of both genes. The older patient's phenotype is more likely caused only by the *TBC1D24* variant. We believe that more attention should be paid to adolescent and adult-onset hearing loss patients, and more frequent referrals to geneticists are warranted.

Keywords Hearing loss, *TBC1D24*, *STRC*, Massive parallel sequencing, Case report

Background

Hearing loss (HL) is the most common sensory deficit, with an estimated prevalence of one to two per 1000 neonates [1–3]. The number increases to almost three per 1000 school-age children and three and a half per 1000 adolescents associated with late-onset or progressive genetic conditions [2, 4]. Approximately 70% of prelingual HL is genetic [2], and most children are diagnosed via various newborn screening programs. HL is heterogeneous; however, adolescent and adult patients with postlingual hearing loss undergo genetic examinations less often than younger children [5]. Nevertheless, adolescent

*Correspondence:
Radka Kremlíková Pourová
radka.pourova@fnmotol.cz
[1] Department of Biology and Medical Genetics, 2nd Faculty of Medicine, Charles University in Prague and University Hospital Motol, Prague, Czech Republic
[2] Department of Otorhinolaryngology and Head and Neck Surgery, 1st Faculty of Medicine, Charles University in Prague and University Hospital Motol, Prague, Czech Republic
[3] Ordinace UNK Louny S.R.O, Louny, Czech Republic
[4] PRENET—Prenatal Diagnostics and Genetics, Laboratoře Lékařské Genetiky s.r.o., Pardubice, Czech Republic

and adult patients also benefit from a molecular-level genetic diagnosis.

Pathogenic variants of the *TBC1D24* gene (OMIM * 613,577) are responsible for five autosomal recessives (AR) early-onset clinical entities, comprising congenital "autosomal recessive deafness 86 (OMIM: 614617)." Pathogenic variants of the *TBC1D24* gene also cause the milder progressive "autosomal dominant deafness 65 (OMIM: 616044)," which develops in the second to third decades of life [6]. Four other autosomal recessive phenotypes linked to pathogenic variants of the *TBC1D24* gene mainly include early-onset intellectual disability and seizures. These phenotypes comprise a continuum with late-onset HL as the mildest and DOORS syndrome (deafness, onychodystrophy, osteodystrophy, mental retardation, and seizures, OMIM: 200500) as the most severe phenotype [7]. Autosomal recessive *TBC1D24* HL was first described on a molecular level in consanguineous Pakistani families by Rehman [8]. Autosomal dominant *TBC1D24* HL was simultaneously described by Zhang and Azaiez [9, 10]. Other non-syndromic *TBC1D24* HL patients with a dominant inheritance pattern have been reported [6, 11], with 57 patients currently described in the literature.

On the other hand, the stereocilin gene *STRC*, also known as DFNB16 (OMIM: 603720), is one of the most common genetic factors causing bilateral mild to moderate HL [12]. This autosomal recessive condition is stable in most patients, not worsening after 50 years of age [13]. *STRC*-related HL is the second most common genetic HL after *GJB2*-related HL (DFNB1A, OMIM 220290). The existence of *STRC* pseudogene, 98% homologous with the main *STRC* gene, complicates the interpretation of sequencing results [14]. The onset of HL is usually in early childhood, generally between 0 and 18 years of age [15]. Deletions of the *STRC* gene are quite common, 1 to 5% in HL patients [16]. These deletions can be isolated or part of deafness-infertility syndrome (OMIM 611102) [17].

In this study, we aimed to diagnose the underlying molecular defect in two patients with hearing loss, a father and son. To further support the pathogenicity of our findings, we correlated the audiological findings with molecular genetic results.

Case presentation

Patient one (P1) was a 51-year-old Caucasian male who consulted the medical genetics department for family planning. At the time, he had no children. At age 15, he experienced a gradual worsening of his hearing and started using hearing aids. P1 was not treated for any other medical conditions. The clinical ENT examination did not detect any additional abnormalities. The audiological examination revealed moderate symmetric sensorineural hearing loss. His hearing thresholds were as follows: 15 dB HL (0.125 kHz), 20 dB HL (0.25 kHz), 30 dB HL (0.5 kHz), 45 dB HL (1 kHz), 50 dB HL (2 kHz), 45 dB HL (3 kHz), 55 dB HL (4 kHz), 65 dB HL (6 kHz), and 60 dB HL (8 kHz) (Fig. 1). P1 has a healthy brother with normal hearing.

His father (P2) was a 76-year-old Caucasian male who had also experienced hearing impairment since high school. He had been intermittently treated with hearing aids. Additionally, he suffers from coronary heart disease

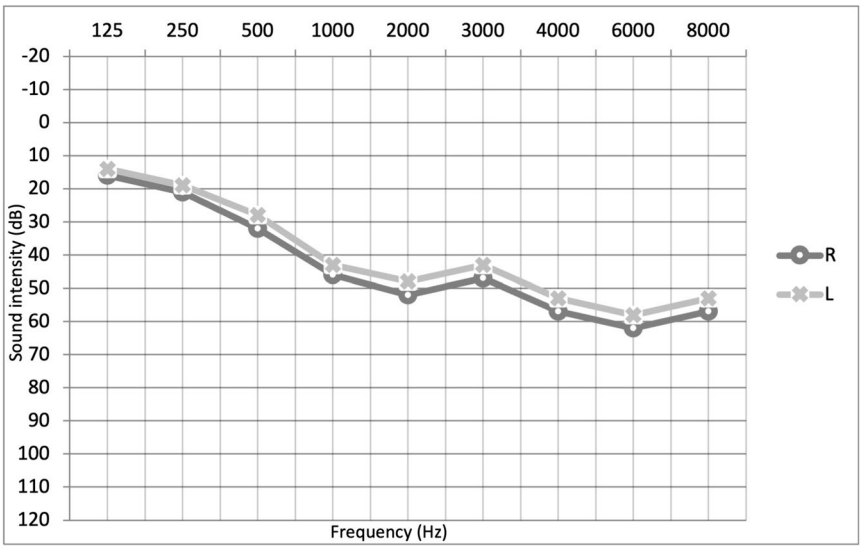


Fig. 1 P1's audiogram

and gastric ulcers. P2 was first examined at a collaborating ENT department at age 59 for worsening hearing and tinnitus. P2 was treated with vasodilating agents and vitamins, which ameliorated his tinnitus. A brain MRI did not detect any signs of acoustic neurinoma. A clinical ENT examination was without any abnormalities. His audiological examination showed moderately severe symmetric sensorineural hearing loss. His hearing thresholds were as follows: 25 dB HL (0.125 kHz), 35 dB HL (0.25 kHz), 39 dB HL (0.5 kHz), 55 dB HL (1 kHz), 57 dB HL (2 kHz), 60 dB HL (3 kHz), 57 dB HL (4 kHz), 67 dB HL (6 kHz), and 62 dB HL (8 kHz) (Fig. 2). No seizures or other symptoms associated with other *TBC1D24* phenotypes were described in either patient. Both patients used hearing aids with excellent results. The progression of hearing loss in P2 may eventually meet the criteria for cochlear implants.

Laboratory methods

Blood samples were collected from P1 and P2, and genomic DNA was extracted from peripheral blood lymphocytes using standard extraction procedures. For *STRC* copy number variant (CNV) detection, a "multiplex ligation-dependent probe amplification" (MLPA) kit number P461 was used (MRC Holland). Samples were initially tested by massive parallel sequencing (MPS, Illumina.com) using a custom-made panel comprising 174 hearing loss-related genes (Roche) (list of genes available in Supplementary material 1), rendering high coverage of all genomic regions of interest. Sequencing data were processed and analyzed using GATK tools, followed by variant prioritization (Variant Interpreter software; Illumina). MPS was not used to detect CNVs. Detected variants were validated using Sanger DNA sequencing and classified according to ACMG guidelines [18].

Afterwards both samples from P1 and P1 were sequenced by SOPHiA Genetics Clinical Exome Solution 3.2 kit (SOPHiA; Switzerland; complete list of genes examined on request from the manufacturer). Sequencing was performed on the NextSeq platform (Illumina; USA) and data were analyzed using SOPHiA DDM software. Detected variants were validated and their segregation in the family was verified using Sanger DNA sequencing.

Audiometric methods

Pure tone audiometry was performed using current standards to determine hearing thresholds at frequencies of 0.25, 0.5, 1, 2, 3, 4, and 8 kHz. Air and bone conduction thresholds were determined to exclude conductive hearing impairment. Patients also underwent a standard clinical ENT examination.

Results

Using MLPA, we initially detected a heterozygous *STRC* gene deletion in both P1 and P2. This deletion was not found in the healthy brother of P1. Since *STRC*-related deafness is an autosomal recessive disorder, and there is a relatively high frequency of (cca 2.6%) heterozygotes in both hearing loss patients and healthy controls [13], plus, no single nucleotide variants in the *STRC* gene were found using subsequent MPS gene panel, we initially concluded that this deletion is highly unlikely to be involved in the hearing loss of our patients.

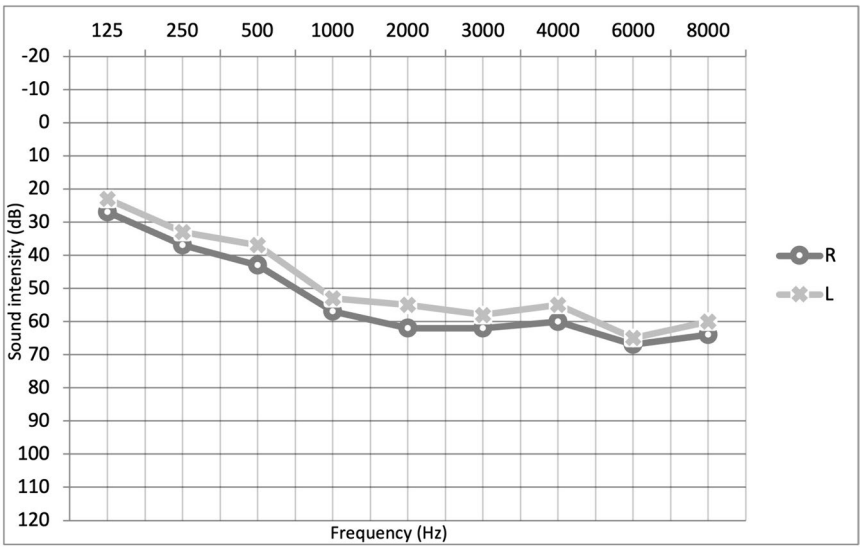


Fig. 2 P2's audiogram

Using the MPS gene panel, we detected the heterozygous variant NM_001199107.1(*TBC1D24*):c.448G > A (p.Glu150Lys) in both father and son (Fig. 3). The SNP RS number of the variant is rs777248380. The variant's frequency in healthy controls is 0.001222% (gnomAD). The variant was previously reported in the ClinVar database as VUS (RCV002287055.2). It is not currently described in the available literature.

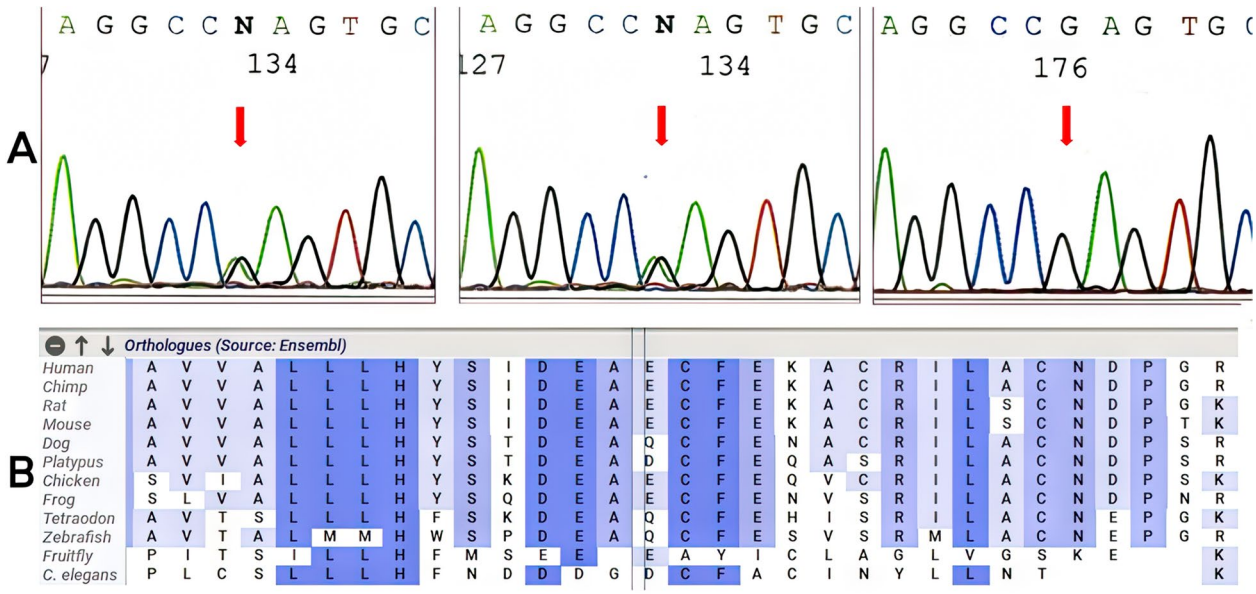
The glutamine residue is moderately conserved; there is a slight physicochemical difference between glutamine and lysine. In silico prediction mechanisms rendered conflicting pathogenicity estimates (Fig. 3). There were no other candidate variants. The variant occurs in a critical protein domain, i.e., Rab-GAP TBC. This variant was not found by targeted Sanger sequencing analysis in a healthy brother of P1 with normal hearing. Based on the American College of Medical Genetics and Genomics (ACMG) classification, we concluded that this variant is of unknown significance (class 3, PM1, PM2, PP2). Nevertheless, based on clinical data and segregation analysis, we suspect the variant is potentially causative for the development of HL in our patients.

Due to the not entirely certain causality of this variant, exome sequencing was subsequently performed for both P1 and P2. The method detected a likely pathogenic indel variant NM_153700.2(*STRC*):c.4917_4918delinsCT p.(Leu1640Phe) in P1. By retrospective data analysis, this indel was also detected in the originally negative MPS panel in P1. It was not reported by the software, most likely because it was in a position next to a second, benign indel variant. This indel variant was not found in P2 nor in the healthy brother of P1. Mother of P1 is not available for genetic testing.

Discussion

Our observations are consistent with published cases, i.e., at diagnosis, cases presented with variable degrees of HL severity, and all authors reported a progressive disease course [6, 8–11]. The limitation of the study is a small number of examined family members for the segregation of the variant and its uncertain nature.

Thus far, the role of the TBC1D24 protein is not well understood. It contains multiple domains implicated in various functions [6]. It is expressed in the spiral ganglion neurons and outer hair cells of the inner ear, as well as



C

Gene	Variant cDNA Level	Variant Protein Level	Exon	Reference SNP ID	Population frequencies			Pathogenicity predictions						ACMG Classification
					gnomAD v2.1.1	UK10K	EVS	SIFT	Polyphen v2.2.8	Mutation Taster	LRT	CADD v1.6	Revel	
TBC1D24 NM_001199107.2	c.448G>A	p.Glu150Lys	2	rs777248380	*f* = 0.0000122	0	0	T (0.05)	B (0.446)	B (Tree vote: 12|88)	D (0.000023)	D (23.3)	B (0.104)	VOUS (PM1, PM2, PP2)

Fig. 3 **A** Sanger DNA sequencing chromatograms—from left to right: P1, P2, healthy brother of P1. **B** Amino acid alignment across species—the amino acid is moderately conserved—Glu is present in 7/12 species. The amino acid is marked with double blue lines under the middle red arrow. **C** In silico predictions comparison. Table adapted from (6). Legend: B benign, D damaging/deleterious, T tolerated, VOUS a variant of unknown significance

in the brain, kidney, heart, and liver [9, 19]. We lack the capacity to run functional studies on this variant, but we are open to collaborations if the opportunity arises. Our variant, p.Glu150Lys, is in the "Rab-GAP TBC" protein domain. Previously detected variants p.Ser178Leu [6, 9, 10] and p.Asp185Asn [6] were also in the same protein domain, although at different amino acid residues. Other previously detected variants, p.His487Gln, p.His487Leu [6], and p.Asn307His [11], are located in a different part of the protein. Interestingly, the mutated *TBC1D24* does not cause HL in a mouse model [20], and most *TBC1D24* AR allelic epileptic disorders do not include HL.

Other reported variants in this gene show a clear progressive worsening of hearing over 10-year timelines with an average loss of 10 dB per decade [6]. P1 and P2, despite a 25-year age difference, had similar audiograms. If we calculate the PTA average for P1, we see that he is losing 1.1 dB per year. The same calculation for P2 gives a result of 0.6 dB per year. These rates of deterioration are quite similar to published rates. We would therefore expect a more significant difference in hearing loss between the two audiograms. A possible explanation would be that each patient's hearing loss might have started at a slightly different age, and progression could have been variable. This hints at a possible intrafamilial, intergenerational, or interfamilial variation in the natural course of the disease. Influence of the *STRC* variants in P1 cannot be ruled out either.

Mild but progressive HL, with a later onset, is almost certainly underdiagnosed, and genetic examinations of these patients are warranted, ideally using robust genome sequencing methods. It would be interesting to observe the effect of our variant in homozygous or compound heterozygous constellations. So far, no such patients have been reported. Identifying HL patients with new variants is essential to facilitate proper genetic counseling. Newly identified variants may one day be used in targeted genetic therapies.

Conclusions

We present two patients with genetic HL. In P2, we strongly suspect HL is based on a variant in the *TBC1D24* gene. In P1, we assume that HL is probably based on the combination of a variant in the *TBC1D24* gene and two variants in the *STRC* gene in trans position. Two variants in the *STRC* gene alone would suffice to cause HL in P1. Possible influence of variants in both genes in one patient has not yet been studied. The *TBC1D24* gene has been previously implicated in various genetic forms of HL. Reports of *TBC1D24* HL genotype–phenotype correlations are still lacking, and we believe data from our patients will further the understanding of *TBC1D24* HL.

Both patients presented with isolated hearing loss, i.e., without any other related health issues. Their HL began in their teenage years and was moderately severe in both patients. In P2, HL had progressed to borderline severe. Such a depth and progression of HL is not typical for *STRC* HL [13]. Had it not been for the atypical constellation of two indel variants in the *STRC* gene, compound heterozygosity in the *STRC* gene would have been detected in H1 by the initial MPS panel examination and MLPA studies. In our case, the diagnosis was made by exome sequencing. In P1, the clinical picture is atypical for HL caused purely by pathogenic variants in *STRC* gene; we assume a concordance of effects of both *STRC* and *TBC1D24* genes. This case further demonstrates the importance of exome sequencing, since panel sequencing initially failed to detect the indel variant in the *STRC* gene.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43163-024-00661-x.

Supplementary Material 1.

Acknowledgements
We want to thank Tom Secrest for English proofreading. We would also like to thank our patients and their families for their cooperation. This work was done under the auspices of ERN ITHACA and CRANIO.

Authors' contributions
MS conceived, designed, drafted the manuscript, and led genetic consultations with the patients. RKP performed molecular genetic laboratory testing and interpreted its results. KH and JB lead otorhinolaryngologic consultations and data collection. MH substantively revised the manuscript. All authors have agreed to be personally accountable for their contributions. All authors read and approved the final manuscript.

Funding
Agentura Pro Zdravotnický Výzkum České Republiky (NW24J-06-00119). Grant AZV number NW24J-06–00119.

Availability of data and materials
The datasets used and/or analyzed during this study are available from the corresponding author.

Declarations

Ethics approval and consent to participate
The case used a standard informed consent procedure, including standing ethics approval from the Internal Ethics Board of the Motol University Hospital for patients diagnosed at our national tertiary genetics center.

Consent for publication
Patients have signed informed consent for publication.

Competing interests
The authors declare no conflict of interest that could affect the content or outcomes reported in this manuscript.

Received: 13 May 2024 Accepted: 23 August 2024
Published online: 04 October 2024

References

1. Komínek P, Chrobok V, Zeleník K, Držata J (2017) Novorozenecký screening sluchu–význam, současný stav v ČR. Čas Lék Čes 156:173–177
2. Morton CC, Nance WE (2006) Newborn hearing screening—a silent revolution. N Engl J Med 354(20):2151–2164. https://doi.org/10.1056/NEJMra050700
3. Smith RJH, Bale JF, White KR (2005) Sensorineural hearing loss in children. Lancet Lond Engl 365(9462):879–890. https://doi.org/10.1016/S0140-6736(05)71047-3
4. Fortnum HM, Summerfield AQ, Marshall DH, Davis AC, Bamford JM (2001) Prevalence of permanent childhood hearing impairment in the United Kingdom and implications for universal neonatal hearing screening: questionnaire based ascertainment study. BMJ 323(7312):536–540. https://doi.org/10.1136/bmj.323.7312.536
5. Ahmadmehrabi S, Brant J, Epstein DJ, Ruckenstein MJ, Rader DJ (2021) Genetics of postlingual sensorineural hearing loss. Laryngoscope 131(2):401–409. https://doi.org/10.1002/lary.28646
6. Oziębło D, Leja ML, Lazniewski M et al (2021) TBC1D24 emerges as an important contributor to progressive postlingual dominant hearing loss. Sci Rep 11(1):10300. https://doi.org/10.1038/s41598-021-89645-y
7. Mucha BE, Hennekam RC, Sisodiya S, Campeau PM. TBC1D24-related disorders. In: Adam MP, Ardinger HH, Pagon RA, et al., eds. *GeneReviews®*. University of Washington, Seattle; 1993. http://www.ncbi.nlm.nih.gov/books/NBK274566/. Accessed 3 Mar 2022
8. Rehman AU, Santos-Cortez RLP, Morell RJ et al (2014) Mutations in TBC1D24, a gene associated with epilepsy, also cause nonsyndromic deafness DFNB86. Am J Hum Genet 94(1):144–152. https://doi.org/10.1016/j.ajhg.2013.12.004
9. Azaiez H, Booth KT, Bu F et al (2014) TBC1D24 mutation causes autosomal-dominant nonsyndromic hearing loss. Hum Mutat 35(7):819–823. https://doi.org/10.1002/humu.22557
10. Zhang L, Hu L, Chai Y, Pang X, Yang T, Wu H (2014) A dominant mutation in the stereocilia-expressing gene TBC1D24 is a probable cause for non-syndromic hearing impairment. Hum Mutat 35(7):814–818. https://doi.org/10.1002/humu.22558
11. Parzefall T, Frohne A, Koenighofer M et al (2020) A novel variant in the TBC1D24 lipid-binding pocket causes autosomal dominant hearing loss: evidence for a genotype-phenotype correlation. Front Cell Neurosci 14:585669. https://doi.org/10.3389/fncel.2020.585669
12. Han S, Zhang D, Guo Y, Fu Z, Guan G. Prevalence and characteristics of STRC gene mutations (DFNB16): a systematic review and meta-analysis. Front Genet. 2021;12. Accessed 5 Oct 2023. https://www.frontiersin.org/articles/10.3389/fgene.2021.707845.
13. Yokota Y, Moteki H, Nishio SY et al (2019) Frequency and clinical features of hearing loss caused by STRC deletions. Sci Rep 9(1):4408. https://doi.org/10.1038/s41598-019-40586-7
14. Francey LJ, Conlin LK, Kadesch HE et al (2012) Genome-wide SNP genotyping identifies the stereocilin (STRC) gene as a major contributor to pediatric bilateral sensorineural hearing impairment. Am J Med Genet A 158A(2):298–308. https://doi.org/10.1002/ajmg.a.34391
15. Markova TG, Alekseeva NN, Mironovich OL et al (2020) Clinical features of hearing loss caused by STRC gene deletions/mutations in Russian population. Int J Pediatr Otorhinolaryngol 138:110247. https://doi.org/10.1016/j.ijporl.2020.110247
16. Plevova P, Paprskarova M, Tvrda P, Turska P, Slavkovsky R, Mrazkova E (2017) STRC deletion is a frequent cause of slight to moderate congenital hearing impairment in the Czech Republic. Otol Neurotol 38(10):e393. https://doi.org/10.1097/MAO.0000000000001571
17. Zhang Y, Malekpour M, Al-Madani N et al (2007) Sensorineural deafness and male infertility: a contiguous gene deletion syndrome. J Med Genet 44(4):233–240. https://doi.org/10.1136/jmg.2006.045765
18. Richards S, Aziz N, Bale S et al (2015) Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 17(5):405–423. https://doi.org/10.1038/gim.2015.30
19. Rehman A, Friedman T, Griffith A (2017) Unresolved questions regarding human hereditary deafness. Oral Dis 23(5):551–558. https://doi.org/10.1111/odi.12516
20. Tona R, Lopez IA, Fenollar-Ferrer C et al (2020) Mouse models of human pathogenic variants of TBC1D24 associated with non-syndromic deafness DFNB86 and DFNA65 and syndromes involving deafness. Genes 11(10):E1122. https://doi.org/10.3390/genes11101122

Publisher's Note