Received: 24 June 2020 | Revised: 24 August 2020 | Accepted: 24 August 2020

DOI: 10.1111/cge.13839

Check for updates

CLINICAL GENETICS WILEY

ORIGINAL ARTICLE

Spectrum and frequencies of non *GJB2* gene mutations in Czech patients with early non-syndromic hearing loss detected by gene panel NGS and whole-exome sequencing

Dana Safka Brozkova[1] | Simona Poisson Marková[1] | Anna Uhrová Mészárosová[1] | Ján Jenčík[1] | Vlasta Čejnová[2] | Zdeněk Čada[3] | Jana Laštůvková[2] | Dagmar Rašková[4] | Pavel Seeman[1]

[1]DNA Laboratory, Department of Paediatric Neurology, Second Faculty of Medicine, Charles University and Motol University Hospital, Prague, Czech Republic

[2]Department of Medical Genetics, Masaryk Hospital in Usti nad Labem, Regional Health Corporation, Usti nad Labem, Czech Republic

[3]Department of Otorhinolaryngology and Head and Neck Surgery, First Faculty of Medicine, Charles University, Motol University Hospital, Prague, Czech Republic

[4]Centre for Medical Genetics and Reproductive Medicine Gennet, Prague 7, Czech Republic

Correspondence
Dana Safka Brozkova, DNA laboratory, Department of Paediatric Neurology, Second Faculty of Medicine, Charles University and Motol University Hospital, V Úvalu 84, 150 06, Praha 5, Czech Republic.
Email: dana.brozkova@lfmotol.cuni.cz

Funding information
Ministry of Health of the Czech Republic, Grant/Award Numbers: AZV 16-31173A, DRO 00064203

Abstract

Non-syndromic autosomal recessive hearing loss is an extremely heterogeneous disease caused by mutations in more than 80 genes. We examined Czech patients with early/prelingual non-syndromic, presumably genetic hearing loss (NSHL) without known cause after *GJB2* gene testing. Four hundred and twenty-one unrelated patients were examined for *STRC* gene deletions with quantitative comparative fluorescent PCR (QCF PCR), 197 unrelated patients with next-generation sequencing by custom-designed NSHL gene panels and 19 patients with whole-exome sequencing (WES). Combining all methods, we discovered the cause of the disease in 54 patients. The most frequent type of NSHL was DFNB16 (*STRC*), which was detected in 22 patients, almost half of the clarified patients. Other biallelic pathogenic mutations were detected in the genes: *MYO15A*, *LOXHD1*, *TMPRSS3* (each gene was responsible for five clarified patients, *CDH23* (four clarified patients), *OTOG* and *OTOF* (each gene was responsible for two clarified patients). Other genes (*AIFM1*, *CABP2*, *DIAPH1*, *PTPRQ*, *RDX*, *SLC26A4*, *TBC1D24*, *TECTA*, *TMC1*) that explained the cause of hearing impairment were further detected in only one patient for each gene. *STRC* gene mutations, mainly deletions remain the most frequent NSHL cause after mutations in the *GJB2*.

KEYWORDS

hearing loss, next-generation sequencing, non *GJB2* patients, non-syndromic hearing loss

1 | INTRODUCTION

Hearing loss with a prevalence of 1 to 2 in 1000 newborns is the most frequent sensory disorder.[1] Hereditary causes account for hearing loss in almost two-thirds of hearing-impaired patients.[2] The often-used approach, which is to divide hearing loss into syndromic and non-syndromic group,[3] is not always so clear since not all of the syndromic signs of deafness may be expressed at the time of diagnosis. An example is the hearing disorder DFNB4, also known as Pendred syndrome (OMIM: 274600), which is caused by biallelic mutations in the *SLC26A4* gene. Patients with biallelic pathogenic mutations in the *SLC26A4* suffer from congenital hearing loss, but most of them develop thyroid enlargement (goitre) with or without hypothyroidism later in adolescence.[4]

The genetic causes of early hearing impairment are most frequently autosomal recessive, named also DFNB, and are responsible for hearing loss in almost 80% of patients.[2] In almost 40% of early hearing loss patients, biallelic causal mutations in the *GJB2* gene are

 © 2020 John Wiley & Sons A/S . Published by John Wiley & Sons Ltd

detected (type DFNB1).[5,6] Therefore, Sanger sequencing of the *GJB2* gene remains a fundamental, fast and cost-effective diagnostics for early non-syndromic hearing loss. In several countries, the large deletion involving the *GJB6* gene has been detected as the frequent cause of hearing loss.[7] The deletion *GJB6*-D13S1830 was mainly detected in compound heterozygous state with *GJB2* mutations. Czech patients with only one heterozygous, monoallelic mutations in *GJB2* were tested for the large 340 kb deletion (*GJB6*-D13S1830) and this deletion was not detected in any of them.[8] Therefore, no second similarly important causal gene besides *GJB2* has been detected yet. It seems

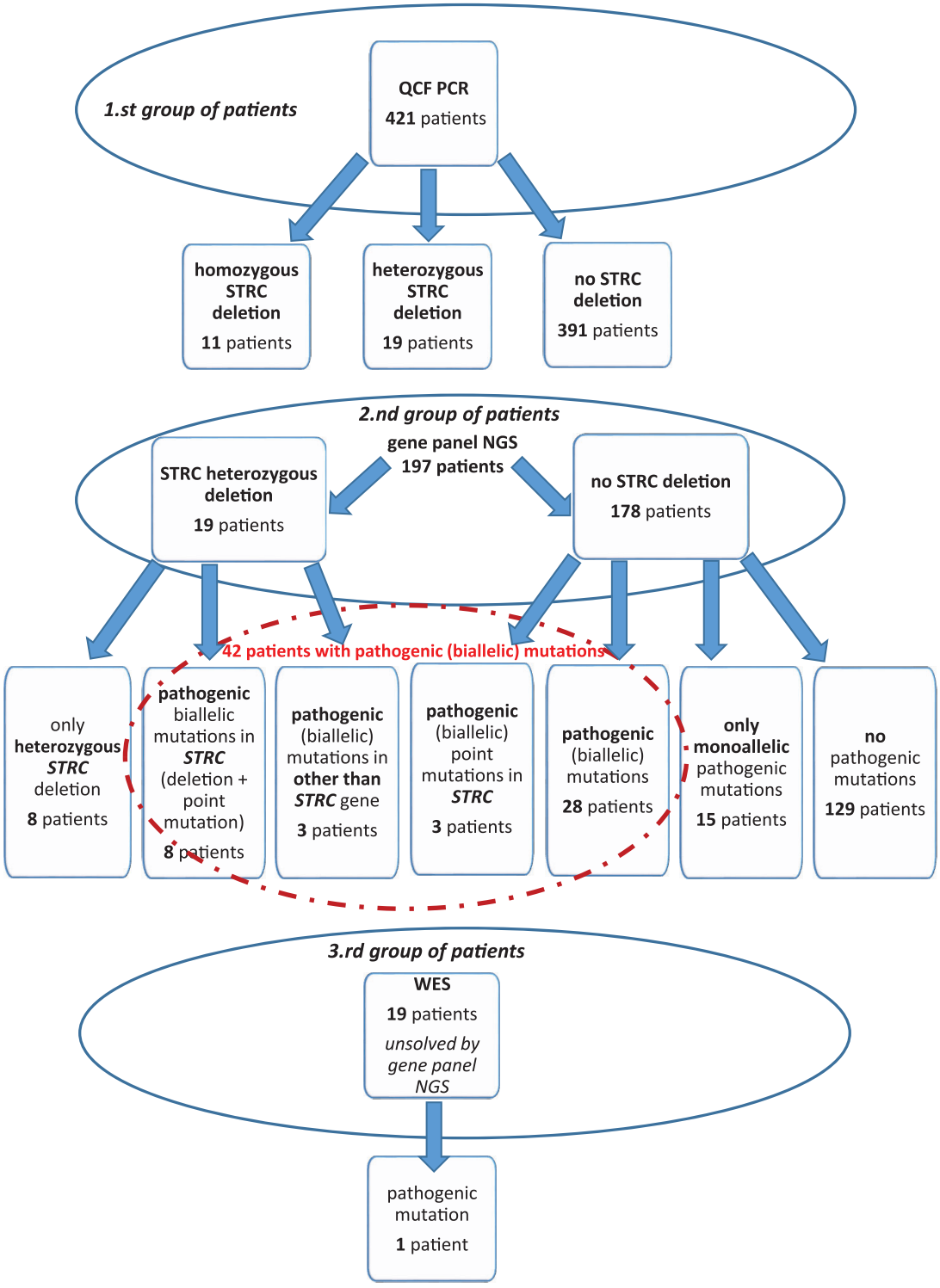


FIGURE 1 Flowchart of examinations. The figure describes the examination procedure in the detection of found variants and the number of genetically confirmed patients for individual methods [Colour figure can be viewed at wileyonlinelibrary.com]

that there are many genes that are collectively responsible for hearing loss in patients that do not have bialellic mutations in the *GJB2* gene.

The use of next-generation sequencing (NGS) has expanded the possibility of diagnostics by examining many genes associated with hearing loss in parallel. We showed that among hearing loss patients without the *GJB2* mutations, nearly, 6% of patients carried biallelic *STRC* gene mutations and thus their type of hearing impairment could be specified to the type DFNB16.[9] We have shown further that Sanger sequencing of eight small *DFNB* genes does not explain the hearing loss in a reasonable number of patients.[10] Similar results were published for part of the Czech Republic,(Moravian-Silesian population) in which no pathogenic mutations were found in *SERPINB6*, *TMIE*, *COCH*, *ESPN*, *ACTG1*, *KCNQ4* or *GJB3*.[11] To date, the genes involved and the spectrum of mutations in early hearing loss Czech patients are largely unknown. We present the largest group of Czech early hearing loss patients (197), where biallelic mutations in *GJB2* had already been excluded, that were examined by gene panel NGS consisting of genes associated with recessive hearing impairment (DFNB) and supplemented by whole-exome sequencing (WES) in 19 of them. Overall, we have clarified hearing impairment in 54 patients and we present the spectrum of mutations and genes associated with early non-syndromic hearing loss in Czech patients.

2 | MATERIALS AND METHODS

All patients were referred and selected by clinical geneticists and were selected on suspicion of genetic hearing impairment presenting early within the first few years of life and having no other or better explanation for the hearing loss (severe prematurity, perinatal risks, very low birth weight, meningitis, so on). All patients fulfilled the criteria for GJB2 gene testing (bilateral, sensorineural, non-syndromic, prelingual or early hearing loss) and were examined prior to this study. Most patients were sporadic but some of them had one or even more siblings with hearing loss—these were familial cases (Table S1). Patients without biallelic mutations in *GJB2* were divided into three consecutive groups (Figure 1). In the first group, consisting of 421 patients, the *STRC* gene deletions were examined with our own quantitative comparative fluorescent polymerase chain reaction (QCF PCR) method described by Markova et al.[9] In the second group, 197 patients were examined with NGS of a gene panel containing hearing loss genes. In the third group, 19 patients were examined with WES. All patients or their representatives signed the informed consent with genetic testing of hearing loss. The study was approved by the ethics committee of the University Hospital Motol.

Genomic DNA from unrelated patients was examined as follows: Patients with no pathogenic *GJB2* mutations (in coding exon 2 and non-coding exon 1) were examined with QCF-PCR[9] for the presence of *STRC* gene deletions. Detected deletions were verified by multiplex-ligation probe amplification with Probemix SALSA P461-DIS (MLPA, MRC-Holland, NL) in all patients. From patients with or without heterozygous *STRC* deletions, 197 patients were selected and examined with NGS of a gene panel. Six versions of the custom-designed gene panel were used and, in each version, the list of genes was updated with novel genes; the gene panel comprised mainly autosomal recessive *NSHL* genes. The number of genes in each version of the panel was: V1-41, V2-67, V3-70, V4-75. V5-79, V6-84 (Table S2). HaloPlex kit (Agilent Technologies, Santa Clara, CA) and SureSelect Target Enrichment kit (Agilent Technologies, Santa Clara, CA) were used for library construction and sequenced on the MiSeq and Hi-Seq platforms (Illumina, Inc., San Diego, CA). From patients without pathogenic mutations after the gene panel NGS, 19 patients were selected for WES and examined with the Human All Exon V6 kit (Agilent Technologies, Santa Clara, CA). The NGS data was analysed with NextGene (Softgenetics, State College, PA) and SureCall (Agilent Technologies, Santa Clara, CA) softwares. The verification of detected variants was performed by Sanger sequencing and in available family members, a segregation analysis was performed. The presence of point mutations in the *STRC* gene was verified by Sanger sequencing from an *STRC* gene-specific product generated by long-range PCR (LR-PCR) with no contamination of the *pSTRC* pseudogene as described by Vona et al.[12]

The NGS data analysis focused on single nucleotide variants (SNV), indels and copy number variants (CNV). We performed the evaluation of variants as follows: the call for variants should be at least 20 and the frequency in public databases (GnomAD, ExAC) should not be above 0.5%. Variants presented in more than three samples per panel were excluded. All remaining variants were evaluated regarding the values of prediction programs (SIFT, Mutation Taster, Polyphen), conservation scores, already published variants and ACMG criteria.

The audiograms were completed from the clinical documentation of patients, where it was available.

3 | RESULTS

The cause of the hearing loss through the use of the methods described either alone or in combination, was clarified in 54 unrelated patients affected by prelingual non-syndromic hearing loss with previously excluded *GJB2* biallelic pathogenic mutations. Considering the gene panel NGS alone (in 197 patients), the cause of hearing loss was clarified in 42 patients, that is, 21% of all gene panel NGS-examined patients (Figure 1).

The most common type of hearing loss was DFNB16 (*STRC*), detected in 22 patients, almost half of all clarified patients regardless of the type of examination. Eleven patients with homozygous (biallelic) *STRC* deletions were detected with QCF PCR. Eight patients with heterozygous *STRC* deletions had the second pathogenic mutation as a sequence variant detected by the gene panel NGS and three patients had biallelic point mutations in *STRC* detected also by the gene panel NGS (Table 2). Interestingly, in three hearing loss heterozygotes for the *STRC* deletion we detected not a second *STRC* pathogenic mutation, but biallelic pathogenic variants in other genes, namely in MYO15A (two patients) and LOXHD1, therefore, these patients are only by chance heterozygous carriers of an *STRC* gene

TABLE 1 An overview of genes with biallelic pathogenic mutations detected by gene panel next-generation sequencing in 42 clarified patients

Gene	Type of hearing loss	Patients with pathogenic biallelic mutations (% of clarified patients)
STRC	DFNB16	11 (27%)
LOXHD1	DFNB8/10	5 (12%)
MYO15A	DFNB3	5 (12%)
TMPRSS3	DFNB77	5 (12%)
CDH23	DFNB12	4 (10%)
OTOG	DFNB18	2 (5%)
OTOF	DFNB9	2 (5%)
AIFM1	AUNX1/ DFNX5	1 (2.5%)
CABP2	DFNB93	1 (2.5%)
PTPRQ	DFNB84	1 (2.5%)
RDX	DFNB24	1 (2.5%)
SLC26A4	DFNB4 / PDS	1 (2.5%)
TBC1D24	DFNB86	1 (2.5%)
TECTA	DFNB21	1 (2.5%)
TMC1	DFNB7/11	1 (2.5%)
All		42 (100%)

TABLE 2 Types of *STRC* biallelic mutations detected by quantitative comparative fluorescent polymerase chain reaction and by gene panel next-generation sequencing

Type of mutation	Number of patients—22 total
Homozygous deletion	11 (50%)
Deletion + point mutation	8 (36%)
Two point mutations	3 (14%)

deletion (Table S1). Additional relevant and important genes, particularly in the diagnostics of Czech patients, are *MYO15A*, *LOXHD1* and *TMPRSS3*, where we detected frequent pathogenic mutations with each explaining the cause of hearing impairment in five patients (12% of clarified patients for each gene) (Table 1, Table S1). Biallelic pathogenic mutations in *CDH23* were found in four patients (10% of clarified patients), *OTOF* and *OTOG* both in two patients (5% of clarified patients). Mutations in another eight genes (*AIFM1*, *CABP2*, *PTPRQ*, *RDX*, *SLC26A4*, *TBC1D24*, *TECTA*, and *TMC1*), which explained the cause of hearing loss, were detected in only one patient or family for each gene.

WES of 19 patients with an initial negative result from the gene panel NGS clarified the cause of hearing loss in only one family. The *DIAPH1* mutation which is responsible for autosomal dominant (AD), early and severe hearing loss was detected as de novo in the mother, whose parents have normal hearing and her daughter with hearing loss and clarified the cause of their hearing loss. The parenthood test by STR markers confirmed the correct parentity. (Table S1, Figure 2).

Interestingly, in one family two different causes of hearing loss were detected in two siblings with hearing loss, namely DFNB3 (*MYO15A*) and DFNB16 (*STRC*). The gene panel NGS detected two possibly pathogenic *MYO15A* mutations in proband with heterozygous *STRC* deletion and clinically severe hearing loss (Table S1). Her sibling with mild/moderate hearing loss carried only one *MYO15A* pathogenic allele, but she is homozygous for the *STRC* deletion. Their parents were without hearing impairment and both were carriers of an *STRC* deletion and one pathogenic *MYO15A* mutation.

Audiological examination of a patient with an *OTOG* nonsense homozygous mutation (p.Gln834*) (4788), that was also detected in a similarly affected brother (171607), showed moderate hearing loss with a pure tone average (PTA) 45 dB at the age of 26 years and 43.3 dB in his sibling (21 years) (Figure 2). In patient 545 509 with probable pathogenic *LOXHD1* mutations, an audiological examination at the age of 12 years revealed profound hearing loss with a PTA 80 dB (Figure 2).

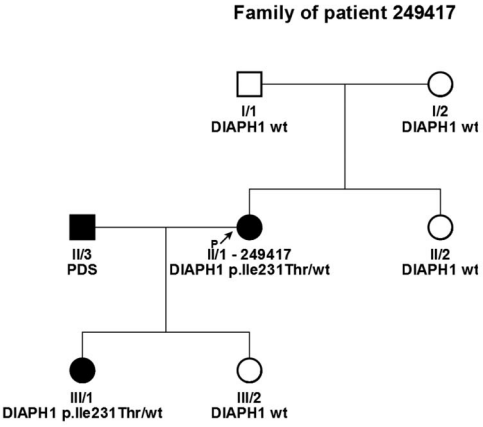
4 | DISCUSSION

Gene panel NGS examination clarified the cause of hearing loss in 21% of examined patients (42 of 197), which may seem like a low number, however, it should be noted that the examined group is reduced further since patients with pathogenic biallelic *GJB2* mutations representing about 40% of patients with an early cause of hearing loss[5] were previously excluded. In 15 patients (7.6%), we detected only one pathogenic heterozygous mutation, which alone could not explain the cause of hearing loss (Table S1). It thus remains unclear whether these patients are only, by chance, carriers of the found pathogenic variants and the cause of hearing loss is in another gene or if there is a second pathogenic mutation in the same gene that had not been discovered using the current methods.

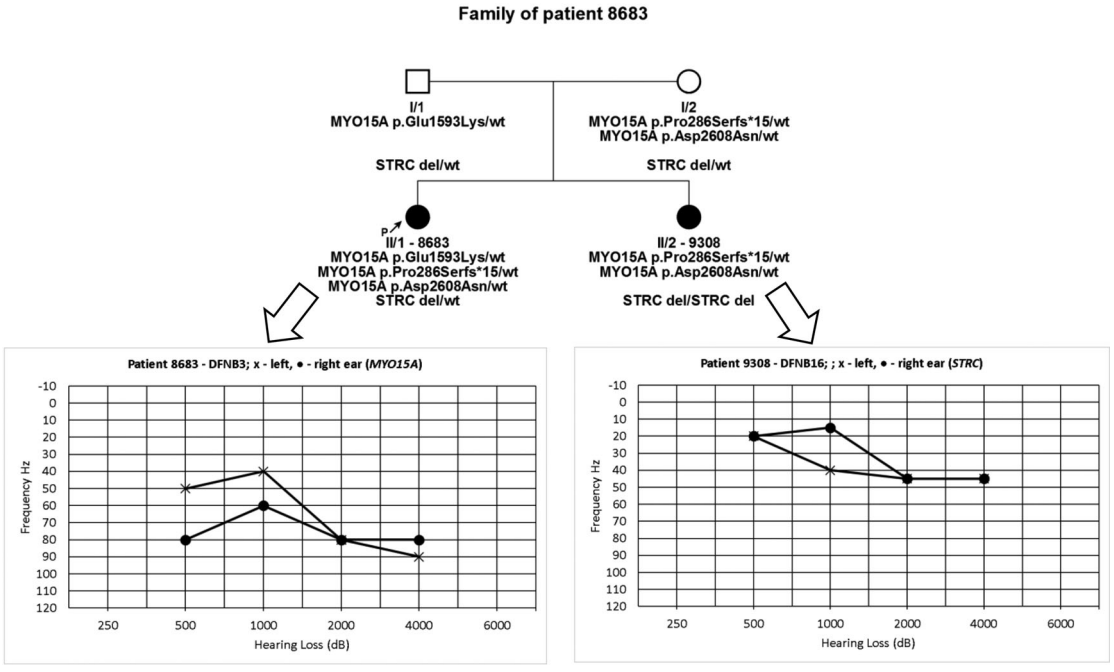
The second most common cause of early hearing loss after *GJB2* mutations seem to be biallelic mutations in the *STRC* gene.[9] Almost half the clarified patients in our study have pathogenic mutations in the *STRC* gene. Importantly, gross deletions are the most frequent and were detected on 19 out of 44 alleles in 22 DFNB16 patients. For this reason, we consider our procedure, performing a QCF PCR examination of *STRC* deletions before the NGS examination, as very advantageous and effective. Patients with a homozygous *STRC* large deletion were easily clarified without the need for NGS examination and we immediately identified the *STRC* heterozygotes despite the fact that the *STRC* gene has a high homology to the pseudogene and CNV analysis of gene panel NGS data is complex for the *STRC* region. In contrast, QCF PCR directly uses the quantification of gene and pseudogene sequence comparison.[9]

In 42% of heterozygotes for *STRC* gene deletion, a second pathogenic mutation was detected on the opposite (undeleted) allele and this finding modifies our previous report by Markova et al,[9] where we found a second explaining mutation in up to 64% of hearing loss heterozygotes for *STRC* gene deletion. This difference probably occurred by chance, due to an uneven distribution of patients with an *STRC* heterozygous deletion in both studies and in the group analysed by Markova et al where more DFNB16 patients with both *STRC*

A – Family of patient 249417 - pedigree



B – Family of patient 8683 – pedigree, audiograms



C – Audiograms

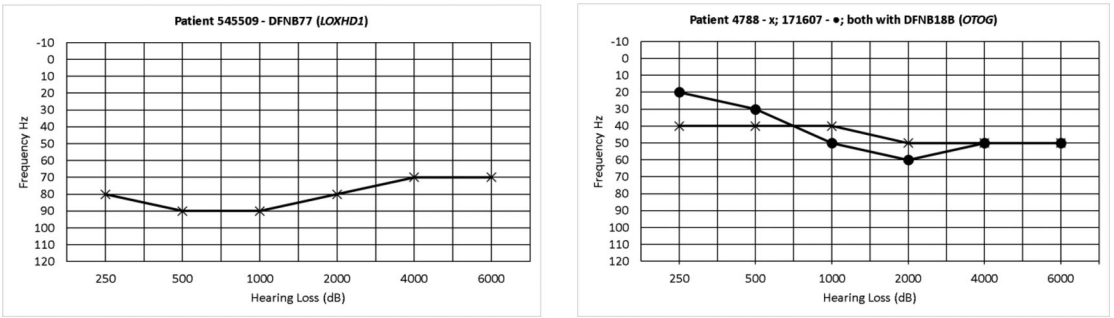


FIGURE 2 wPedigrees + audiograms. A, Pedigree for family of patient 249 417 where the autosomal dominant *DIAPH1* variant was detected in two generations and originated de novo in proband 249 417. Pedigree for family of patient 8683, where we detected two different causes of hearing loss in two siblings. The biallelic pathogenic mutations in *MYO15A* (DFNB3) were detected in proband 8683, who is only a heterozygous carrier for the *STRC* gene deletion and her audiogram (C) showed severe hearing loss. The proband's sister 9308 is only a carrier of the pathogenic *MYO15A* mutation and her hearing loss is moderate due to a homozygous deletion of the *STRC* gene (DFNB16). B, Audiograms of available patients. For each gene/type of hearing loss, there is a different degree of hearing loss shown

pathogenic mutations were included. The *STRC* gene deletions were recently reported as frequent cause of mild to moderate hearing loss in some countries. In the group of *GJB2* negative patients with mild to moderate hearing loss *STRC* biallelic mutations were reported in 5.5% and 6% of patients in the United States and Germany.[12,13]

In three *STRC* gene deletion heterozygotes, the cause of hearing loss was detected in genes other than *STRC* and thus are only random carriers of *STRC* deletions. This is consistent with the report by Knijnenburg et al, who, by screening of healthy control individuals and a review of publicly available CNV data, estimated the frequency of heterozygous deletion carriers to be about 1.6%.[14]

The question is: why was the cause of hearing loss not clarified in eight patients with a heterozygous *STRC* deletion? We assume that the second pathogenic variant in the *STRC* gene may not have been captured, or that they may be only random carriers of the *STRC* deletion. The aetiology of hearing loss for these patients may lie in another genetic mutation or in a gene yet to be identified.

In the selected 19 patients, in whom the cause of hearing loss remained unexplained after the gene panel NGS, a subsequent WES examination clarified the cause of hearing loss in only one patient (5%). This may show, that gene panel NGS detects most of the relevant causes of DFNB and the add on value of WES is only limited. The detected mutation by WES was a de novo heterozygous mutation in the *DIAPH1* gene, which is associated with an autosomal dominant type DFNA1 hearing loss. Unlike other DFNAs, DFNA1 hearing loss is early and severe, as is typical and known for autosomal recessive (DFNB) hearing loss.[3] However, because the gene panel NGS focused mainly on recessive hearing loss, the *DIAPH1* gene was not included. Given that the benefit of WES was only 5% (1/19), as we are suggesting in this study, the examination of predominantly DFNB genes in early-onset hearing loss patients seems to be sufficient for clarification in most cases.

The family with two different causes of hearing loss shows the importance of audiological examination and the correlation of genotype to phenotype in interpreting the detected variants (Figure 2). Both siblings had a different severity of hearing loss, which was finally explained by genetic analysis. DFNB16 manifests as a moderate hearing loss,[15] whereas mutations in the *MYO15A* gene causing DFNB3 result in profound hearing loss,[16] and we have shown these different audiograms in correlation with detected mutations in this family (Figure 2). Audiograms in two siblings with DFNB18B show a moderate hearing loss with a slightly sloping down audiogram as was reported by Schraders et al in the initial report about the *OTOG* mutations.[17] The audiogram from the patient with DFNB77 caused by the *LOXHD1* mutations shows profound hearing loss. The patient reported early onset of his hearing loss, which is in contrast to the first report about DFNB77 where it occurred at the age of 7–8 years and progressed to become moderate to severe at mid and high frequencies during adulthood.[18] However, our findings were consistent with Edvardson et al.,[19] who reported that patients with DFNB77 suffer from severe to profound congenital nonprogressive nonsyndromic hearing loss. Comparison of the clinical findings, especially the audiograms, is very important for correct interpretation of detected variants and confirming the pathogenicity of the genetic mutations.

We present a complex overview of mutations detected by gene panel NGS and WES in patients with non *GJB2* early hearing loss that is the first such extensive report for Czech patients. We highlight that the most common and important genes after the *GJB2* are *STRC*, *MYO15A*, *LOXHD1*, *TMPRSS3* and *CDH23* and they can explain hearing loss in 14% of the total number of 21% clarified Czech patients examined by gene panel NGS. However, the question remains, why there are still so many unexplained patients with presumably genetic and early onset hearing loss (79% of NGS panel examined patients). A similar study, that focused on autosomal recessive NSHL, and included patients from Western Europe, showed that the frequently mutated genes were *TMC1*, *MYO7A* and *MYO15A*, where biallelic mutations elucidate 9% of 22% clarified patients by gene panel NGS.[20] Thus, it turns out that other genes play an important role in hearing loss in patients from Central Europe, as we have shown by examining the Czech population. The question remains whether, given the comparable prices of gene panel NGS and WES, it is more appropriate to use WES as the primary method since with WES all known genes are included and not only genes for autosomal recessive NSHL. Our results, based on a limited number of patients, showed that the clarification of WES compared to gene panel NGS is of little benefit, yet the advantage of WES is that it allows the data to be re-evaluated whenever a new cause of hearing loss appears but without the need to repeat or expand the examination. There is a risk that pathogenic variants in genes that are not related to hearing impairment will be captured, but this ethical issue must be addressed through informed consent before the examination.

We envisaged our results to improve substantially the genetic diagnostics in early non-syndromic hearing loss not only in the Czech population, but in other populations, too. Knowledge of causal variants and the exact cause of hearing loss will aid precise genetic counselling with an exact genetic prognosis for the patients and their relatives. It will also offer families targeted genetic prevention in terms of preimplantation diagnostics, appropriate treatment as well as enable patients to plan for any future family.

ACKNOWLEDGEMENTS

The authors thank the patients and families for participating in the study and to physicians for their cooperation and examination of patients. Supported by the Ministry of Health of the Czech Republic, AZV 16-31173A and DRO 00064203. All rights reserved.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/cge.13839.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

ORCID

Dana Safka Brozkova https://orcid.org/0000-0002-0572-7239
Anna Uhrová Mészárosová https://orcid.org/0000-0002-6186-5142

REFERENCES

1. Morton CC, Nance WE. Newborn hearing screening—a silent revolution. *N Engl J Med*. 2006;354(20):2151-2164.
2. Marazita ML, Ploughman LM, Rawlings B, Remington E, Arnos KS, Nance WE. Genetic epidemiological studies of early-onset deafness in the U.S. school-age population. *Am J Med Genet*. 1993;46(5):486-491.
3. Gorlin RJ, Toriello HV, Cohen MM Jr. *Hereditary Hearing Loss and its Syndromes*. New York: Oxford University Press; 1995.
4. Wemeau JL, Kopp P. Pendred syndrome. *Best Pract Res Clin Endocrinol Metab*. 2017;31(2):213-224.
5. Seeman P, Malikova M, Raskova D, et al. Spectrum and frequencies of mutations in the GJB2 (Cx26) gene among 156 Czech patients with pre-lingual deafness. *Clin Genet*. 2004;66(2):152-157.
6. Seeman P, Sakmaryova I. High prevalence of the IVS 1 + 1 G to a/GJB2 mutation among Czech hearing impaired patients with monoallelic mutation in the coding region of GJB2. *Clin Genet*. 2006;69(5):410-413.
7. Del Castillo I, Moreno-Pelayo MA, Del Castillo FJ, et al. Prevalence and evolutionary origins of the del(GJB6-D13S1830) mutation in the DFNB1 locus in hearing-impaired subjects: a multicenter study. *Am J Hum Genet*. 2003;73(6):1452-1458.
8. Seeman P, Bendova O, Raskova D, Malikova M, Groh D, Kabelka Z. Double heterozygosity with mutations involving both the GJB2 and GJB6 genes is a possible, but very rare, cause of congenital deafness in the Czech population. *Ann Hum Genet*. 2005;69(Pt 1):9-14.
9. Markova SP, Brozkova DS, Lassuthova P, et al. STRC gene mutations, mainly large deletions, are a very important cause of early-onset hereditary hearing loss in the Czech population. *Genet Test Mol Biomarkers*. 2018;22(2):127-134.
10. Markova S, Safka Brozkova D, Meszarosova A, et al. Mutations in eight small DFNB genes are not a frequent cause of non-syndromic hereditary hearing loss in Czech patients. *Int J Pediatr Otorhinolaryngol*. 2016;86:27-33.
11. Plevova P, Tvrda P, Paprskarova M, et al. Genetic Aetiology of Non-syndromic hearing loss in Moravia-Silesia. *Medicina (Kaunas)*. 2018;54(2):1-9.
12. Vona B, Hofrichter MA, Neuner C, et al. DFNB16 is a frequent cause of congenital hearing impairment: implementation of STRC mutation analysis in routine diagnostics. *Clin Genet*. 2015;87(1):49-55.
13. Francey LJ, Conlin LK, Kadesch HE, et al. Genome-wide SNP genotyping identifies the Stereocilin (STRC) gene as a major contributor to pediatric bilateral sensorineural hearing impairment. *Am J Med Genet A*. 2012;158A(2):298-308.
14. Knijnenburg J, Oberstein SA, Frei K, et al. A homozygous deletion of a normal variation locus in a patient with hearing loss from non-consanguineous parents. *J Med Genet*. 2009;46(6):412-417.
15. Cada Z, Safka Brozkova D, Balatkova Z, et al. Moderate sensorineural hearing loss is typical for DFNB16 caused by various types of mutations affecting the STRC gene. *Eur Arch Otorhinolaryngol*. 2019;276(12):3353-3358.
16. Friedman TB, Liang Y, Weber JL, et al. A gene for congenital, recessive deafness DFNB3 maps to the pericentromeric region of chromosome 17. *Nat Genet*. 1995;9(1):86-91.
17. Schraders M, Ruiz-Palmero L, Kalay E, et al. Mutations of the gene encoding otogelin are a cause of autosomal-recessive nonsyndromic moderate hearing impairment. *Am J Hum Genet*. 2012;91(5):883-889.
18. Grillet N, Schwander M, Hildebrand MS, et al. Mutations in LOXHD1, an evolutionarily conserved stereociliary protein, disrupt hair cell function in mice and cause progressive hearing loss in humans. *Am J Hum Genet*. 2009;85(3):328-337.
19. Edvardson S, Jalas C, Shaag A, et al. A deleterious mutation in the LOXHD1 gene causes autosomal recessive hearing loss in Ashkenazi Jews. *Am J Med Genet A*. 2011;155A(5):1170-1172.
20. Sommen M, Schrauwen I, Vandeweyer G, et al. DNA diagnostics of hereditary hearing loss: a targeted Resequencing approach combined with a mutation classification system. *Hum Mutat*. 2016;37(8):812-819.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Safka Brozkova D, Poisson Marková S, Mészárosová AU, et al. Spectrum and frequencies of non *GJB2* gene mutations in Czech patients with early non-syndromic hearing loss detected by gene panel NGS and whole-exome sequencing. *Clinical Genetics*. 2020;1–7. https://doi.org/10.1111/cge.13839