European Archives of Oto-Rhino-Laryngology (2019) 276:3353–3358
https://doi.org/10.1007/s00405-019-05649-5

OTOLOGY

Moderate sensorineural hearing loss is typical for DFNB16 caused by various types of mutations affecting the STRC gene

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Received: 11 August 2019 / Accepted: 11 September 2019 / Published online: 24 September 2019

Abstract

Introduction Hearing loss is the most frequent sensory disorder and is genetically extremely heterogeneous. By far the most frequent cause of nonsyndromic autosomal recessive hearing loss (AR-NSHL) are biallelic pathogenic mutations in the *GJB2* gene causing DFNB1. The worldwide search for the second most common type of AR-NSHL took almost two decades. Recently reported alterations (mostly deletions) of the *STRC* gene, also named DFNB16, seem to be the second most frequent cause of AR-NSHL. Genetic testing of STRC is very challenging due to the highly homologous pseudogene. Anecdotal evidence from single patients shows that *STRC* mutations have their typical audiological findings and patients usually have moderate hearing loss. The aim of this study is to discover if audiological findings in patients with biallelic pathogenic mutations affecting *STRC* have the characteristic features and shape of audiological curves and if there are genotype/phenotype correlations in relation to various types of *STRC* mutations.

Methods Eleven hearing loss patients with pathogenic mutations on both alleles of the *STRC* gene were detected during routine genetic examination of AR-NSHL patients. Audiological examination consisted of pure tone audiometry, stapedial reflexes, tympanometry and otoacoustic emission tests.

Results The threshold of pure tone average (PTA) was 46 dB and otoacoustic emissions were not detectable in these DFNB16 patients. All patients were without vestibular irritation or asymmetry.

Conclusion Moderate sensorineural hearing loss is typical for DFNB16-associated hearing loss and there are no significant differences in audiological phenotypes among different types of mutations affecting *STRC*.

Keywords Autosomal recessive nonsyndromic hearing loss · DFNB16 · STRC · Stereocilin · Audiological phenotype · Genotype/phenotype correlation

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Introduction

Hearing loss is the most common sensory deficit and one of the most frequent genetic disorders in humans [1]. The prevalence range is 1:500–1:1000 [1, 2]. The main division of hearing loss is between syndromic and nonsyndromic (NSHL). These two entities can be autosomal recessive with early and more severe hearing loss, dominant, with later and milder hearing loss or X-linked being very rare. Although deafness is genetically very heterogeneous, the most common nonsyndromic autosomal-recessive hearing loss (AR-NSHL), also known as DFNB, is responsible for almost 80% of NSHL patients [3]. At least 70 genes are reported to be responsible for AR-NSHL [4]. Of the next generation sequencing methods, massivelly parallel sequencing of selected genes is the most frequently used and effective approach for the genetic examination of hearing loss in patients without *GJB2* biallelic mutations.

By far the most common cause of AR-NSHL are mutations affecting the locus DFNB1 with the *GJB2* gene [5]. Biallelic pathogenic mutations in the *GJB2* are detectable in almost 40% of Czech prelingual hearing loss patients (AR-NSHL patients) [6]. The second most frequently affected hearing loss locus is DFNB16. Biallelic pathogenic mutations are detected in 5.5% of Czech NSHL patients [7]. Similar results were reported also for patients from Germany where biallelic (homozygous or compound heterozygous) pathogenic mutations affecting *STRC* were detected in 6% of NSHL patients [8].

Homozygous or compound heterozygous biallelic pathogenic alterations of the *STRC* gene (MIM: 606440) are responsible for DFNB16 hearing loss. *STRC* gene encodes a highly conserved protein Stereocilin that ensures the connection of the neighboring stereocilia of the outer hair cells and is also important for linking stereocilia with tectorial membrane [9–11].

STRC is located on chromosome 15q15.3 in a region of complex duplication with three other genes, *PPIP5K1* (MIM:610979), *CATSPER2* (MIM:607249) and *CKMT1A* (MIM: 613415) [12]. Diagnostic testing of *STRC* is challenging due to the presence of a highly homologous pseudogene *pSTRC* (99.6% similarity for coding sequence and 98.9% similarity with intronic sequences). For the diagnostics of deletions, a simple method QCF-PCR using the different lengths of *STRC* and *pSTRC* and quantification of these fragments was implemented for detection in Czech patients [7]. The presence of point mutations detected by next generation short reads sequencing (NGS) should be always verified in the gene specific product by long-range PCR and Sanger sequencing to prevent contamination by *pSTRC* [13].

STRC deletions have a variable extent and frequently involve the neighbouring *CATSPER2* gene which is essential for sperm motility. Clinical unit deafness infertility syndrome in men (DIS-MIM:611102) is therefore used for the *STRC* deletions [14].

Very recently, a publication delineating the phenotype of DFNB16 in nine patients from Germany was published presenting their audiological findings [13]. Here we present a new report from more patients showing hearing loss in 11 Czech DFNB16 patients.

Material and methods

Eleven patients (four male and seven female) with biallelic pathogenic mutations affecting the *STRC* gene (DFNB16 patients) were studied. All patients or their parents signed informed consent with genetic testing of hearing loss. The Ethics Committee of University Hospital Motol in Prague approved the examination. All patients underwent common clinical genetic examination including family history and routine ENT screening consisting of otoscopic view of eardrum under the microscope and audiologic tests including pure tone audiometry, stapedial reflexes, tympanometry and otoacoustic emission tests.

Pure tone air and bone conduction hearing threshold were evaluated by calibrated audiometry (Madsen Astera, Otometrics, DK) in frequencies 250, 500, 1000, 2000, 4000, 6000 Hz. Pure tone average (PTA) was calculated from frequencies 500, 1000, 2000 and 4000 Hz. The level of hearing loss was calculated and graded according to the level of PTA from frequencies 500, 1000, 2000 and 4000 Hz to normal hearing (PTA < 20 dB), mild hearing loss (PTA 21–40 dB), moderate hearing loss (PTA 41–70 dB), severe hearing loss (PTA 71–95 dB) and profound hearing loss (PTA > 95 dB).

Tympanometry and stapedial reflex measurements were performed in each patient with tympanometer (AZ 26, AT 35, Interacoustics, DK) and otoacoustic emission test (TEOAE) was performed by using the tympanometer (ILO 6, Otodynamics, UK) in five patients.

Statistical analysis

Statistical analyses were performed using SPSS version 25 (SPSS Inc., Chicago, IL, USA). Basic descriptive statistics were computed for hearing loss at frequencies 250, 500, 1000 and 4000 Hz. Differences in hearing loss at various frequencies were examined using a Friedman test (an alternative to repeated measures ANOVA) followed by a series of Wilcoxon signed-rank tests. Given the relatively small sample size, we used Monte Carlo resampling procedure with $N = 10{,}000$ samples, which compensate for tied values and do not depend on asymptotic approximations for p

values [15]. *p* values below 0.05 were considered statistically significant.

Genetic testing

Genetic examination of patients with early hearing impairment proceeded as follows. The *GJB2* gene mutations were previously examined by Sanger sequencing (coding exon of *GJB2* and region of c. − 23 + 1 G > A in non-coding exon in heterozygotes for pathogenic mutation in exon 2) [6]. In patients with no *GJB2* mutations, the *STRC* deletions were examined by a simple method of quantitative comparative fluorescent polymerase chain reaction (QCF-PCR) [7]. The verification of detected deletions was performed with multiplex-ligation probe amplification (MLPA, MRC-Holland, NL). Patients without *STRC* deletions and patients with only one (heterozygous) deletion were examined with targeted NGS of a gene panel for early recessive hearing loss. SureSelect target enrichment kit (Agilent technologies, US) was used for library construction and sequenced on the Illumina Hi-Seq platform (Illumina, Inc., US). Fastq NGS data were analyzed with two softwares: NextGene (Softgenetics, US) and SureCall (Agilent technologies, US). The presence of point mutations in the *STRC* gene was verified by Sanger sequencing from a *STRC* gene specific product generated by long range PCR (LR-PCR) with no contamination of *pSTRC* pseudogene as described by Vona et al. [8].

Results

Eleven hearing loss patients with biallelic pathogenic mutations in the *STRC* gene were divided into three groups according to the type of mutations. The largest group of seven patients comprised the homozygous deletions of *STRC* gene. The second group was composed of two patients with heterozygous *STRC* deletion and hemizygous pathogenic mutations (nonsense mutation p.Arg1073*). The third group consisted of two compound heterozygotes for pathogenic point mutations (two different nonsense mutations p.Arg1073* and p.Arg1468*) (Table 1). The study group consisted of four males and seven females in age range from 6 to 37 at the time of examination (Table 1).

The spectrum of detected mutations among the DFNB16 patients is summarized in Table 2. Altogether 23 DFNB16 patients were detected during routine genetic examination. It was possible to perform audiological examination in 11 of them. Almost half of the detected DFNB16 patients are homozygous for *STRC* deletion (11/23 patients). The most frequent mutation is the *STRC* deletion found on 30 alleles (30 of 46). The second most frequent is nonsense mutation p.Arg1073* found on eight alleles and the third most frequent is p.Arg1468* found on three alleles. Surprisingly, almost all the detected mutations affect the length of the final protein with only one exception. A missense mutation p.Arg928Ser changing the one aminoacid to another with probably no impact on the final protein length was detected only in one patient with combination of heterozygous deletion.

Tympanogram type A together with positive stapedial reflexes was observed in all patients. Otoacoustic emission test TEOAE was measured in five patients (1, 2, 3, 8, 9) and the results confirmed the level of hearing loss.

The audiological results of pure tone audiometry showed symmetric mild to moderate sensorineural hearing loss (Figs. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11). Neither conductive nor combined hearing loss was detected; hearing loss is purely sensorineural. Pure tone average (PTA) was 46 dB, median

Table 1 The Overview of the audiologically examined patients with biallelic *STRC* mutations

No		PTA	Allele 1	Allele 2	Interpretation
1	Female, 38 years	50	*STRC* gene deletion	*STRC* gene deletion	Homozygous deletion
2	Female, 10 years	43	*STRC* gene deletion	*STRC* gene deletion	Homozygous deletion
3	Female, 14 years	38	*STRC* gene deletion	*STRC* gene deletion	Homozygous deletion
4	Female, 14 years	50	*STRC* gene deletion	*STRC* gene deletion	Homozygous deletion
5	Male, 19 years	53	*STRC* gene deletion	*STRC* gene deletion	Homozygous deletion
6	Female, 36 years	65	*STRC* gene deletion	*STRC* gene deletion	Homozygous deletion
7	Male, 17 years	43	*STRC* gene deletion	*STRC* gene deletion	Homozygous deletion
8	Female, 15 years	38	c.4402C > T,p.Arg1468*	c.3217C > T, p.Arg1073*	Compound heterozygous for pathogenic point mutations
9	Male, 18 years	40	c.4402C > T,p.Arg1468*	c.3217C > T, p.Arg1073*	Compound heterozygous for pathogenic point mutations
10	Female, 6 years	43	*STRC* gene deletion	c.3217C > T, p.Arg1073*	Heterozygous deletion and hemizygous pathogenic point mutation
11	Male, 13 years	43	*STRC* gene deletion	c.3217C > T, p.Arg1073*	Heterozygous deletion and hemizygous pathogenic point mutation

The age is at time of examination. The pure tone average (PTA) was calculated from frequencies 500, 100, 200, 4000 Hz. The type of mutation is specified for each allele

Table 2 The spectrum of detected *STRC* mutations in the DFNB16 patients

	Allele 1	Allele 2	Interpretation	Possible causality of the genetic change	Number of patients (23 in total)
A	STRC gene deletion	STRC gene deletion	Homozygous deletion	No gene product	11
B	STRC gene deletion	c.3217C > T,p.Arg1073*	Heterozygous deletion and hemizygous pathogenic point mutation	Premature stop mutation — probably no gene product	5
C	c.3217C > T,p.Arg1073*	c.4402C > T,p.Arg1468*	Compound heterozygous for pathogenic point mutations	Premature stop mutation— probably no gene product	3
D	STRC gene deletion	c.2171_2174del, p.Val724Glyfs*6	Heterozygous deletion and hemizygous pathogenic point mutation	Premature stop mutation— probably no gene product	1
E	STRC gene deletion	c.875 + 1G > A, p.?	Heterozygous deletion and hemizygous pathogenic point mutation	Splice mutation probably affecting the gene product length	1
F	STRC gene deletion	c.2784G > T, p.Arg928Ser	Heterozygous deletion and hemizygous pathogenic point mutation	Missense mutation—probably not affecting the gene product length	1
G	c.3502-3503del, p.Gln1168Valfs*30	c.3502-3503del, p.Gln1168Valfs*30	Homozygous for pathogenic point mutation	Premature stop mutation— probably no gene product	1

The possible impact of the detected mutations on the protein level is similar for all except the F. The change for F is a heterozygous deletion combined with missense mutation affecting the sequence of the protein, but not the length of the final protein

43 dB. Average hearing loss in the following frequencies 250, 500, 1000 and 4000 Hz is 37, 42, 52 and 46 dB respectively (Fig. 12).

In terms of the shape of the curves, the cup-shaped deflection of the auditory curves is evident in 8/11 patients at mid-frequencies (Figs. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10). The statistical analysis of PTA did not reveal any significant differences among patients with different types of mutations (CNVs or point mutations) affecting *STRC* (Fig. 12).

Discussion

Here we present a unique group of 11 patients with biallelic *STRC* mutations in correlation with audiological examination. The group of eleven Czech DFNB16 examined patients enlarged and supported the previous study similarly focused on DFNB16 phenotypes published by Back et al. this year [13]. Although the *STRC* gene was firstly reported as causal for hearing impairment in 2001 [11], its high similarity with pseudogene *pSTRC* makes its genetic detection very complicated. Effective diagnosis of *STRC* mutations has been implemented only in the last four years using MLPA, QCF-PCR and LR-PCR for verifying variants only in the gene sequence [7].

Symmetric mild to moderate sensorineural hearing loss was detected in all Czech DFNB16 patients. No difference was found on hearing gains depending on the age in our study, which correlates with the results in available literature [13].

However, at first glance (Figs. 1, 8) it can be confirmed that elderly patients (patients 1 and 8) have a decrease of about 10–15 dB in speech frequencies and in lower frequencies such as patient 8. OAE were absent in these two patients. TEOAE were also absent in patients 1, 2, 3, 8, 9. In other patients, the emissions were either not measured or the data obtained from this examination were not available. Although emissions have not been measured in all patients, it can be stated that their inefficiency correlates with the results of available studies [13]. The cup-shaped deflection of the auditory curves measured in our patients was the most frequent finding. This phenotype was not observed in Back et al. study (9).

The genetic character of detected mutations formed three groups: (1) homozygous deletion (2) heterozygous deletion and hemizygous for pathogenic mutation and (3) compound heterozygous pathogenic point mutations. There was no unambiguous/significant difference in audiological examination among all groups from our study. In comparison with our results, Back et al. proved a marked difference between compound heterozygotes for nonsense and missense mutations in the degree of hearing impairment and its development during the time. Compound heterozygous patients with a nonsense mutation had hearing loss across all frequencies while patients with a missense mutation showed affected frequencies primarily limited to the main speech field [13]. On the contrary, the similar audiological findings among all three our groups could be related to the character of detected mutations. Both nonsense mutations p.Arg1073* and p.Arg1468* form a premature stop codon which could

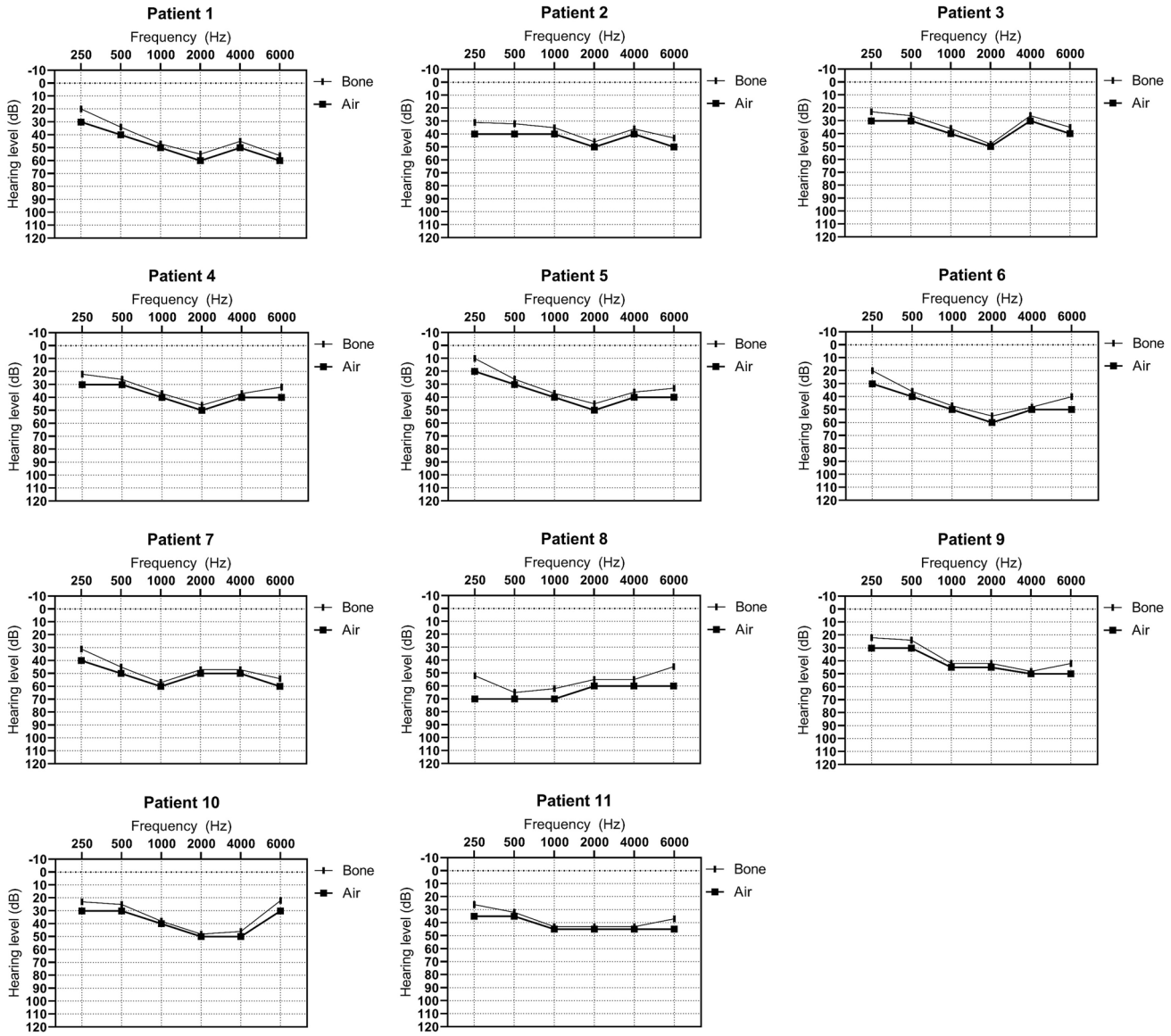

Fig. 1 to 11 Audiograms of all patients. Bone and air hearing thresholds are presented. The cup-shaped deflection of the auditory curves at mid-frequencies is evident in patients 1, 2, 3, 4, 5, 6, 7, 10

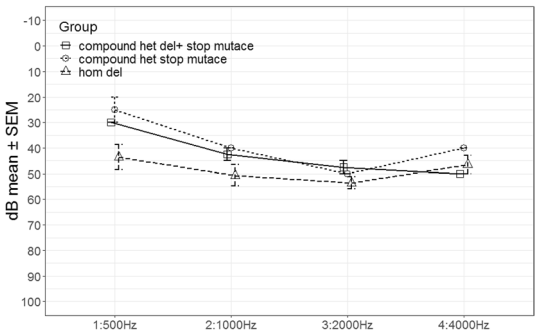

Fig. 12 The audiological mean of PTA among patients with different types of mutations

form a shorter final protein and as the protection from this consequences could be a target for Nonsense mediated decay (NMD) process. NMD leads to a destruction of the shorter mRNA, affected by the nonsense mutation. Results could be very similar in patients with homozygous *STRC* deletions where the absence of deleted DNA from both alleles will result in no protein product. In patients from the second group combining the one allele deletion and nonsense mutation of *STRC* the pathological mechanism will probably be similar leading to NMD on the second allele of *STRC*.

Moreover, the spectrum of detected *STRC* mutations clearly shows that the character of mutations is rated to have a severe impact on the protein level in 22 of 23 DFNB16

patients. Therefore, it was not possible to recruit patients with biallelic missense mutations to include all the spectrum of genetic mutations to this study. At the same time, it also shows that similar composition in terms of mutation types will be found elsewhere in the world and a similar phenotype can be expected in other DFNB16 patients.

Conclusion

DFNB16 hearing loss can be audiologically characterized as a moderate sensorineural hearing loss. Proper genetic diagnostics should include detection of *STRC* deletions subsequently completed by Sanger sequencing of *STRC* gene specific product for point mutations in patients with hemizygous or no deletion of *STRC*. This is only the second study dealing with genotype/phenotype correlations of various biallelic pathogenic mutations affecting the *STRC* gene.

Acknowledgments Supported by the Ministry of Health of the Czech Republic, Grant no. 16-31173A and DRO 00064203.

Compliance with ethical standards

Conflict of interest The authors have no competing interests to declare.

Informed consent Research involving Human Participants who signed informed consent. Informed consent has been signed by all participants.

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