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Mutations in eight small DFNB genes are not a frequent cause of non-syndromic hereditary hearing loss in Czech patients

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ABSTRACT

Objectives: To evaluate the contribution of eight small NSHL-AR (non-syndromic deafness, autosomal recessive) genes to hereditary hearing loss in Czech patients.
Patients and Methods: Unrelated Czech patients, adults and children, diagnosed with pre-lingual hereditary hearing loss with at least one similarly affected deaf sibling and with previously excluded mutations in the *GJB2* gene were investigated by Sanger sequencing of the selected eight small NSHL-AR associated genes (*CABP2* - 51 patients, *CIB2* - 45 patients, *PJVK/DFNB59* - 53 patients, *GJB3* - 46 patients, *ILDR1* - 48 patients, *LHFPL5* - 66 patients, *LRTOMT* - 60 patients, *TMIE* - 64 patients).
Results: Mutations were detected in the *LHFPL5* (DFNB67) gene. The patient is heterozygote for two already described pathogenic variants (p.Tyr127Cys, p.Thr165Met). In five samples, five rare heterozygous variants (two novel) predicted as pathogenic were detected in genes *CABP2, ILDR1, LHFPL5* and *LRTOMT*.
Conclusion: Mutations in eight small NSHL-AR genes are not a frequent cause of hereditary hearing loss in the Czech Republic. This diagnostic approach permitted the clarification of HL in only one patient – two heterozygous mutations were detected in *LHFPL5* gene for the first time in Central Europe. As the use of panel base MPS certainly improves the diagnostic yield, future studies should rather profit from that diagnostic strategy.

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1. Introduction

Hearing loss (HL) is the most prevalent sensory disorder and bilateral, sensorineural hearing loss of 40 dB or more has an estimated incidence of at least 1.33 per 1000 newborns [1]. Genetic HL accounts for more than 50% of them. It is mostly non-syndromic (in 70%) and transmitted as an autosomal recessive (75–80%) trait [2].

In the Czech Republic, NSHL-AR (non-syndromic deafness, autosomal recessive) is predominantly caused by mutations in the *GJB2* gene–coding for connexin 26 (more than 40%) with c.35delG accounting for more than 80% of pathogenic mutations [3]. The large 309 kb deletion involving *GJB6* was detected only once and is probably very rare in central Europe [4], however a splice site mutation in the non-coding part of the *GJB2* gene IVS1 + 1G>A represents the third most frequent pathogenic mutation in *GJB2* in the Czech population [5].

Despite numerous analyses in more than 50% of Czech patients, the cause of NSHL-AR remains unknown.

In this study we Sanger sequenced all coding exons of eight small NSHL-AR genes with up to eight exons, associated with NSHL-AR– *CABP2, CIB2, DFNB59, GJB3, ILDR1, LHFPL5, LRTOMT, TMIE* (Table 1) to estimate their contribution to NSHL in Czech patients.

2. Patients and methods

2.1. Patients enrollment

In total, seventy-two independent Czech patients, adults and children, diagnosed with pre-lingual NSHL were included in the study. Fifty of them have normally hearing parents, 12 have one affected parent and 1 has affected both parents. In 9 patients the hearing status of the parents is not known.

DNA samples from 51 independent patients were investigated by Sanger sequencing of *CABP2* gene, 45 independent patients of *CIB2* gene, 53 independent patients of PJVK/*DFNB59* gene, 46 independent patients of *GJB3* gene, 48 independent patients of *ILDR1* gene, 66 independent patients of *LHFPL5* gene, 60 independent patients of *LRTOMT* gene, and 50 independent patients of *TMIE* gene.

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Table 1
Selected 8 genes description*.

	Gene symbol	DFNB type	Reference sequence	Number of Exons		Chromosomal locus	Number of mutations associated with HL*	Alternative titles; symbols; associated proteins	Year of description	Original article	Geographic origin of described family/families
				All	Coding						
1.	*CABP2*	DFNB93	NM_016366.2	7	7	11q13.1	3	Ca2 + -binding proteins related to calmodulin (Aliases: Calcium-binding protein 2)	2012	[6]	Iran
2.	*CIB2*	DFNB48	NM_006383	6	6	15q24	8	Calcium and integrin binding family member 2 (Aliases: Calcium and integrin-binding family member 2; DNA-dependent protein kinase catalytic subunit-interacting protein 2)	2012	[7]	Pakistan, Turkey
3.	*GJB3*	-	NM_024009.2	2	1	1p34.3	22	Gap junction protein, beta 3, 34 kDa (connexin 31) (Aliases: Connexin 31; Connexin-31; Gap junction beta-3 protein)	1998	[8]	China
4.	*ILDR1*	DFNB42	NM_001199799.1	8	8	3q13.33	17	Immunoglobulin-like domain containing receptor 1 (Aliases: Immunoglobulin-like domain-containing receptor 1; Immunoglobulin-like domain-containing receptor 1 alpha; Immunoglobulin-like domain-containing receptor 1 beta)	2011 (2005)	[9]	Pakistan, Iran, Korea
5.	*LHFPL5*	DFNB67	NM_182548.3	4	3	6p21.31	8	Lipoma hmgic fusion partner-like 5 (Aliases: LHFP-like protein 5; Lipoma HMGIC fusion partner-like 5 protein; Tetraspan membrane protein of hair cell stereocilia)	2005/2006	[10–12]	Pakistan, Turkey, India, Tunisia
6.	*LRTOMT*	DFNB63	NM_001145308.1	7	5	11q13.4	11	Leucine rich transmembrane and 0-methyltransferase domain containing (Aliases: Leucine rich transmembrane and 0-methyltransferase domain containing; Transmembrane O-methyltransferase)	2008	[13]	Tunisia, Turkey, Pakistan, Iran
7.	*PJVK*	DFNB59	NM_001042702.3	7	6	2q31.2	18	Deafness, autosomal recessive 59 (Aliases: Autosomal recessive deafness type 59 protein; Pejvakin-group gasdermins)	2006	[14]	Iran, Turkey, Palestine, Morocco
8.	*TMIE*	DFNB6	NM_147196.2	4	4	3p21.31	10	Transmembrane inner ear expressed gene (Aliases: Transmembrane inner ear expressed protein; Transmembrane inner ear protein)	2002	[15]	Pakistan, India, Turkey

* source HGMD 11.04.2016.

All participating individuals involved in this study signed an informed consent for DNA testing for clarification of their hearing loss.

2.1.1. Inclusion criteria

The inclusion criteria were non-syndromic deafness, being tested previously negative for pathogenic mutations in coding part of *GJB2*, and at least one sibling additionally affected.

2.1.2. Exclusion criteria

The exclusion criteria included suspected syndromic deafness and clearly dominant inheritance in three or more subsequent generations.

2.2. Gene selection

Eight genes (*CABP2, CIB2, DFNB59, GJB3, ILDR1, LHFPL5, LRTOMT, TMIE*) were selected according to the 3 following criteria: associated with NSHL-AR, less than eight coding exons, and no previous analyses of them in the Czech population.

Further description (including NM reference sequence number) of selected genes is summarized in Table 1.

2.3. Molecular genetic analysis

DNA was isolated from whole blood by standard techniques.

All coding exons and intron boundaries of the selected eight genes were Sanger sequenced with a set of 37 pairs of PCR primers (Appendix: Supplementary File 1) designed by ExonPrimer [16]. The resulting PCR products were sequenced with the Big Dye Terminator v3.1 kit and analyzed on the ABI3130 Genetic Analyzer.

Sequence chromatograms were analyzed by Sequencing Analysis software accompanied by MutationSurveyor [17].

2.4. In-silico analysis of known and novel variants

Exome Aggregation Consortium (ExAC) [18], ClinVar [19] and dbSNP databases The Human Gene Mutation Database: Building a comprehensive mutation repository for clinical and molecular genetics, diagnostic testing and personalized genomic medicine [38] and Deafness Variation Database [39] were used to evaluate the frequency and significance of the identified variants.

Alamut visual [20] was used to screen through the detected variants. PolyPhen2, SIFT and MutationTaster [21–23] software were used to predict pathogenicity of the rare known or novel exonic variants. Intronic variants were evaluated by Alamut Visual incorporated programs (ESE Finder [24], RESCUE ESE [25], Human Splicing Finder [26], SpliteSiteFinder [27], MaxEntScan [28], GeneSplicer [29], NNSPLICE [30]). Chance for exon-skipping was evaluated by EX-SKIP [31].

3. Results

The cause of deafness was clarified only in one case – the patient is a heterozygote for two previously described pathogenic mutations in *LHFPL5*.

No known pathogenic biallelic mutations were detected in other genes. However, in five samples, rare variants in heterozygous state predicted as damaging were identified (Table 2), two of them novel.

Detected intronic and exonic variants evaluated as benign/likely benign are summarized in the Appendix: Supplementary File 2.

3.1. LHFPL5

Two described pathogenic mutations in heterozygous state were detected in sample 6056_11, in exon 1: rs104893975, c.380 A > G, p.Tyr127Cys, and in exon 2: rs104893976, c.494 C > T, p.Thr165Met.

The first mutation (p.Tyr127Cys – Fig. 1) was originally described in a family from Chennai, India [10]. The second mutation (p.Thr165Met – Fig. 2) was originally detected in a large consanguineous Turkish family [11].

Patient 6056_11 was born to normally hearing parents and was diagnosed as deaf shortly after his birth. He has two similarly affected deaf brothers and one normally hearing brother. His child and children of his siblings are not affected by deafness (Fig. 3). We expect the patient is a compound heterozygote with mutations in trans, though, it was not verified by segregation analysis, as DNA samples of the family members were not available.

In the sample 3948_12, a novel heterozygous variant was detected (Table 2).

3.2. CABP2, ILDR1, LRTOMT

In four samples, rare variants predicted as pathogenic were detected on one allele only (Table 2.).

3.3. CIB2,DFNB59/PJVK,GJB3, TMIE

No known pathogenic variants, neither variants with pathogenic prediction, were detected.

4. Discussion

In this study we investigated the contribution of eight NSHL-AR genes (*CABP2, CIB2, DFNB59, GJB3, ILDR1, LHFPL5, LRTOMT, TMIE*) to hearing loss in Czech patients. We Sanger sequenced all coding regions of selected genes in about 50 independent patients (45–66 depending on the gene) with at least one affected sibling anticipating AR inheritance (NSHL-AR is the most common type of HL and most of the patients had both parents unaffected). According to our literature research, these genes were tested for the first time in patients from the Czech Republic. The number of selected samples of around 50 is representative for detection of a gene which would have an impact on hereditary HL in the Czech population and would be important for further DNA diagnostic testing. This was unfortunately not the case and we clarified the cause of deafness only in one patient and in one gene, despite very careful selection of patients, even with familiar occurrence.

The number of analyzed samples in selected genes varies due to available DNA quantity limitation. Because of the disappointing results it appears more important to retain some DNA samples for further analyses by new methods (e.g. massively parallel sequencing – MPS).

Pathogenic mutations were identified in the *LHFPL5* gene which encodes TMHS (tetraspan membrane protein of hair cell stereocilia). TMHS is present in hair cells of cochlea [32], and participates in mechano-electrical transduction [33].

The clarified patient is probably the first detected heterozygote for two pathogenic mutations in *LHFPL5* in the world. Pathogenic variants have been described in families with Palestinian, Indian, Turkish, Tunisian, Algerian and Iranian origin. Totally, eight variants associated with HL were described (Table 3). The variant p.Thr165Met has been identified in one Turkish family [11] and variant p.Tyr127Cys in a consanguineous family living in Chennai, India and recently in a family from Iran [10,36].

Both variants have comparable allele frequencies in non-Finnish European populations (NFE) (~6.00e-05). Variant p.Tyr127Cys was additionally detected only once in the South Asian population with a frequency corresponding to frequencies in the NFE population. However, variant p.Thr165Met has not been detected in any other population than NFE [18].

Nevertheless, this one positive finding in the *LHFPL5* gene does not mean that this gene would be a more promising candidate for

Table 2
Rare variants predicted as probably/possibly damaging. Inclusion criteria: 1. MAF < 0.0088*; 2. Predicted as damaging by at least 2/3 prediction programs; 3. Less than 2 homozygotes detected.

GENE	Exon/ intron	c.position and nucleotide change	AA change	Allele 1/Allele 2	dbSNP	MAF (primarly source ExAC – NFE, or other)	Homozygotes reported (ExAc)	In-Silico predictions			Clinical significance (ClinVar)	Detected in SAMPLE
								PolyPhen-2	SIFT	MutationTaster		
CAPB2	Exon 3	c.227G > A	p.Arg76Gln	1/0	rs150664916	0.0001826	0	Probably damaging	Tolerated	Disease causing	not found	1470_09
	Exon 5	c.466G > A**	p.Glu156Lys	1/0	rs143579624	0.0000908	1 (NFE)	Probably damaging	Deleterious	Disease causing	not found	2657
ILDR1	Exon 3	c.307G > A	p.Ala103Thr	1/0	rs200130100	0.0005100	0	Probably damaging	Tolerated	Disease causing	not found	6345_12
LHFPL5	Exon 1	c.86G > T	p.Trp29Leu	1/0	not found	not found	not found	Probably damaging	Deleterious	Disease causing	not found	3948_12
LRTOMT	Exon 5	c.249C > G	p.Phe83Leu	1/0	not found	0.0001216	not found	Possibly damaging	Deleterious	Polymorphism	not found	4607_06

* MAF (NFE) equal to the most common causal mutation for NSHL-AR in the Czech population – GJB2, c.35delG.
** this variant has recently been identified in both allelles as likely pathogenic in a patient with profound to moderate prelingual HL in Iran [36].

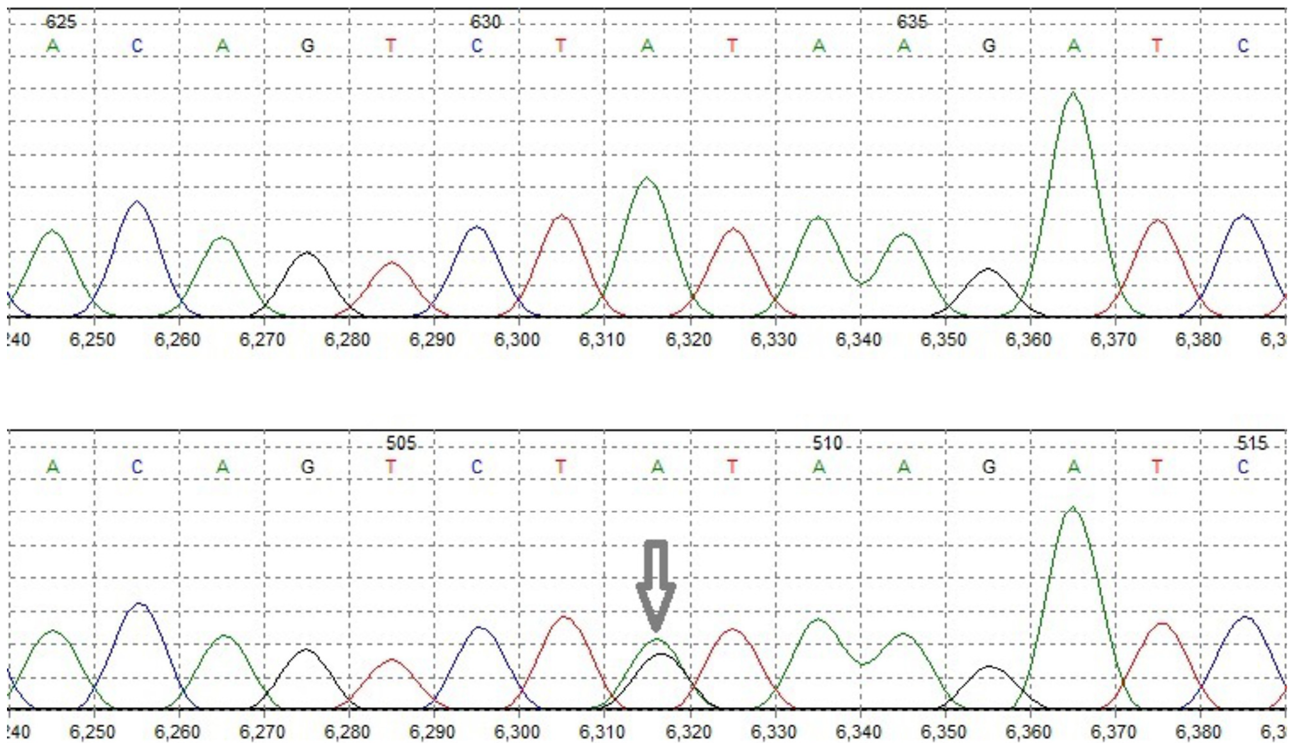

Fig. 1. Sequence chromatograms of patient 6056_11 - *LHFPL5* variant c.380 A > AG, p.Tyr127Cys. Top line – wild type; bottom line – patient.

separate DNA testing. The detection of only one patient may be influenced by chance.

In other genes several novel or rare heterozygous variants on only one allele that may be pathogenic were detected. They are summarized in Table 2. These variants have low MAF and were predicted as probably/possibly damaging, deleterious or disease causing by at least two prediction software programs.

Reliable interpretation of their significance though remains unclear, as no second mutation was found, and the deletions in the remaining exons were not excluded. In the future their significance

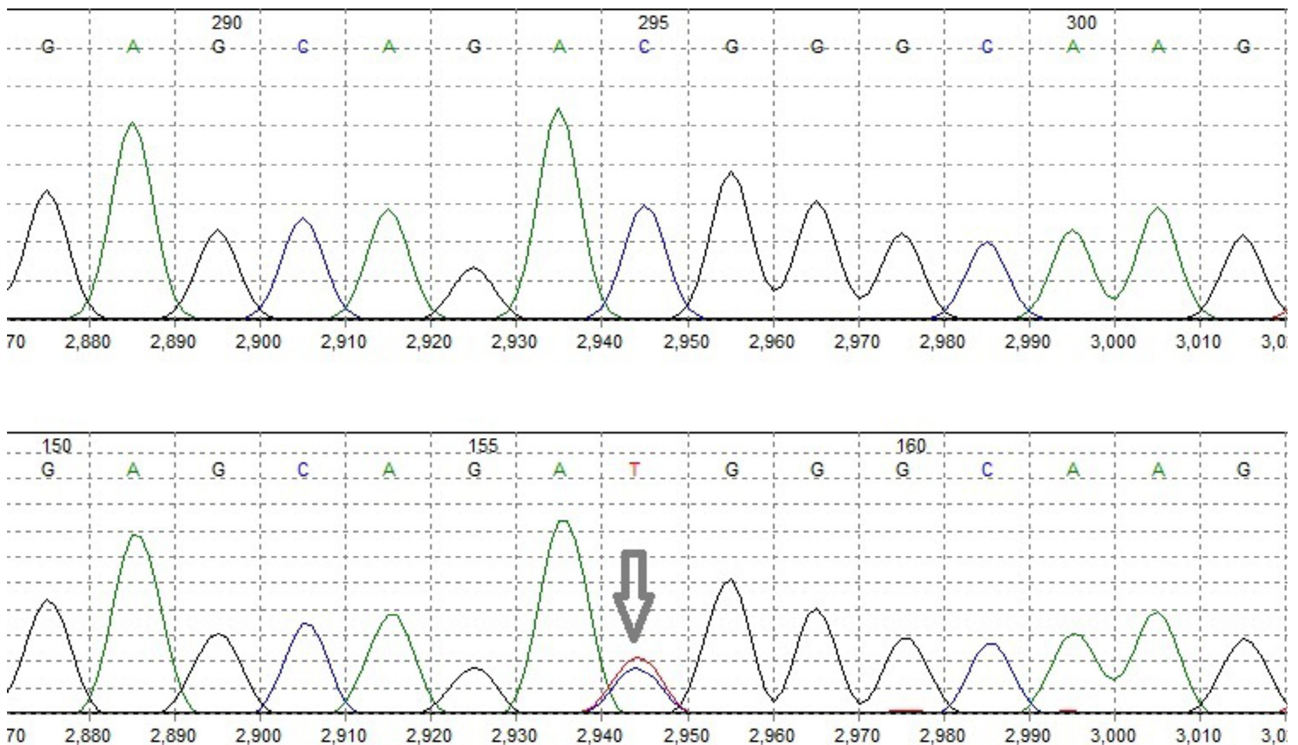

Fig. 2. Sequence chromatograms of patient 6056_11 – *LHFPL5* variant c.494 C > CT, p.Thr165Met. Top line – wild type; bottom line – patient.

Table 3
Summary of yet known pathogenic/with uncertain significance mutations in LHFPL5 associated with HL*.

	Nucleotide change	AA change	c.position and nucleotide change	Origin of the first described family	References
1.	ATG→GTG	Met1Val	c.1A > G	Palestine	[34]
2.	TAT→TGT	Tyr127Cys	c.380A > G	Chennai, India	[10]
3.	ACG→ATG	Thr165Met	c.494C > T	Turkey	[11]
4.	TGC→AGC	p.Cys173Ser	c.518 T > A	Algeria	[35]
5.	AGACTTctcCTCCAT		c.258_260delCTC	Iran	[36]
6.	GGCCcCCTAGACTTC		c.246delC	Pakistan	[10]
7.	ACCGgTAATCACCC		c.649 + 1delG	Turkey	[11]
8.	TGGGGgTACCCTCACC		c.89dupG	Tunisia	[37]

* source HGMD and DVD 20.3.2016.

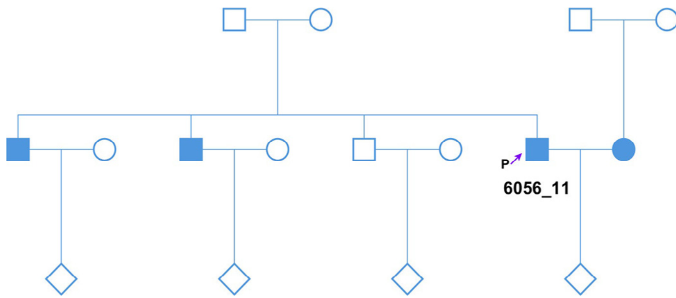

Fig. 3. Pedigree of Czech family (patient 6056_11) affected by DFNB-67 (LHFPL5): Clear symbols – healthy individuals, filled symbols – affected patients. Symbol square – male, symbol circle – female, diamond symbol – gender not specified.

will probably be further evaluated (e.g.MPS and CNV-copy number variation, MLPA for deletion exclusion) and in relevant cases even by functional studies.

5. Conclusion

Selected 8 genes seem to have very low impact on AR NSHL in the Czech population because their analysis clarified the cause of deafness in only one out of around 50 selected patients.

With this approach, we managed to identify the pathogenic mutations in *LHFPL5* and the probable cause of NSHL-AR in only one deaf individual. Our results show that in Czech NSHL patients, it is not reasonable to perform single gene testing of any of these small NSHL-AR genes, nor testing all of them in parallel, as the probability of identifying the cause of hereditary deafness is very low. This may apply also for other surrounding countries.

The use of panel based MPS will certainly improve the diagnostic yield and our future studies will rather profit from that diagnostic strategy.

Conflict of interest

None.

Acknowledgments

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Appendix. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.ijporl.2016.04.005.

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