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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Audiological features in Serbian patients with hearing impairment identified with c.35delG in the *GJB2* gene

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SUMMARY

Introduction/Objective Hearing impairment is the most common sensorineural disorder with an incidence of 1/700–1000 newborns. Variants in the *GJB2* gene are the major cause of autosomal recessive nonsyndromic sensorineural hearing loss (ARNSHL). The degree of hearing impairment in patients with detected mutations in *GJB2* gene ranges from mild to profound.
The aim of this study was to determine possible genotype–phenotype association between audiometric characteristics and detected genotypes in ARNSHL patients from Serbia.

Methods Ninety-two patients with ARNSHL underwent genetic analysis with amplification-refractory mutation system polymerase chain reaction and sequencing of the *GJB2* gene. Audiological analyses were obtained in all patients using a combination of several methods to estimate the degree of hearing loss.

Results Audiological analysis performed in the 92 probands showed moderate to profound range of hearing loss. All identified pathogenic variants accounted for 42.39% of the mutant alleles (78/184 alleles), with the c.35delG mutation being the most frequent one (30.43%). Genotype–phenotype correlation in an isolated group of 37 patients bearing c.35delG in the homozygous, compound heterozygous, or heterozygous state. In this group the majority of patients (30/37, 81.08%) exhibited severe to profound hearing deficit.

Conclusion Association between genotype and the degree of hearing impairment in patients analyzed in this study demonstrated that patients with bi-allelic truncating mutations, i.e., c.35delG, associate with the more severe hearing loss when compared with those identified with only one affected allele. The various degrees of hearing impairment observed in heterozygous patients could be explained by the presence of an undetected second mutation or other modifier genes or environmental causes.

Keywords: hearing impairment; *GJB2* gene; c.35delG variant; audiological features

INTRODUCTION

Hearing loss is the most common sensorineural disorder in humans, with prevalence of one in 700–1000 children and 70% occurs as a nonsyndromic form [1–3]. Approximately half of these cases are genetic in origin [4]. The pattern of inheritance can be distinguished in autosomal recessive (loci named DFNB) (80%), autosomal dominant (DFNA) (17%), X-linked (DFNX) (2–3%), and mitochondrial forms (< 1%) [4].

The *GJB2* gene (the human gap junction β-2 gene - OMIM*121011) which encodes connexin 26 protein, was the first gene that was associated to nonsyndromic sensorineural hearing loss, and mutations in this gene are the most common cause of this disorder in many populations worldwide [1, 3]. More than 200 different pathogenic variants were identified in this gene [5, 6].

Because of the high number of identified variants, their frequency and distribution vary between populations, even within countries [1, 7, 8, 9]. Among these variants, c.35delG frameshift mutation is the most causative mutation of autosomal recessive sensorineural hearing impairment in Caucasians, with an incidence of 70% among the pathogenic alleles and a carrier rate of 1–3% [3, 4, 7–11].

The majority of mutations in connexin 26 are loss of function mutations, resulting in deprived permeability of channel or loss of gap junction and/or hemichannel function [12, 13]. The nonsyndromic hearing loss in patients with mutations in *GJB2* gene ranges from severe to profound and is generally nonprogressive [4, 7, 8].

The purpose of this study was to determine the possible genotype-phenotype association between the audiometric characteristics and the detected genotypes in a group of patients from Serbia with bilateral prelingual non syndromic sensorineural hearing loss.

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METHODS

Patients

Patients were initially clinically evaluated at the Unit for Audiology and Neuro-otology, which is a part of the Department of Otorhinolaryngology at the Dr. Vukan Čupić Mother and Child Health Care Institute of Serbia, during the period from April 2016 to June 2018. All the patients in this study had nonsyndromic bilateral sensorineural hearing impairment which corresponds to autosomal recessive pattern of inheritance (ARNSHL). The analyzed group consisted of 92 patients, 49 males and 43 females. The mean age at diagnosis of patients was 9.5 years, ranging from 3.5 months to 68 years. The protocol of this study was approved by the national Medical Ethics Committee (number 8/20, dated 29.06.2018.), and informed consent was obtained from all patients and/or parents/guardians.

Audiological analysis

Inclusion criteria for patients in the study was nonsyndromic bilateral sensorineural hearing impairment. In each patient, history of hearing loss, family history and physical examination were obtained to determine the age at onset of deafness and to exclude the possibility of environmental causes. Clinical evaluation was done to exclude syndromic hearing impairment by audiologist and pediatrician. Also, tympanometry was performed to exclude conductive hearing impairment. Based on all examinations on patients, it was concluded that patients have nonsyndromic bilateral sensorineural hearing impairment which corresponds to autosomal recessive pattern of inheritance. Children younger than four years (40 patients) underwent transient-evoked otoacoustic emission, brainstem-evoked response audiometry, and auditory steady-state response methods to estimate hearing loss. In addition to the mentioned methods, in patients older than four years (52 patients), the pure-tone audiometry with a diagnostic audiometer in a soundproof room was also done. According to the European protocol, the severity of hearing impairment in probands was classified as mild (20–40 dB), moderate (41–55 dB), moderately severe (56–70 dB), severe (71–95 dB), or profound (more than 95 dB) [4].

DNA analysis

Peripheral blood samples of all patients and their relatives were collected for genetic analysis. DNA analyses were initially performed at the Laboratory of Medical Genetics of the Mother and Child Health Care Institute of Serbia. Sequencing of the coding exon 2 of the *GJB2* gene was performed at the Department of Molecular Genetics, Function and Therapy at the Cyprus Institute of Neurology and Genetics. Genomic DNA was extracted from the peripheral blood samples with ethylenediamine tetraacetic acid using GenJet™ Genomic DNA Purification Kit (ThermoFisher Scientific Inc, Waltham, MA, USA) as instructed by the manufacturer.

Amplification-refractory mutation system polymerase chain reaction for direct detection of c.35delG variant in the GJB2 gene

Direct detection of c.35delG mutation in the *GJB2* gene was carried out in all the patients, using the amplification-refractory mutation system polymerase chain reaction (ARMS-PCR) method, according to modified original method [14, 15]. In two separate PCR reactions, three primers, one common reverse, one normal forward, and one mutant forward were used (size of amplicon at 202 bp). As an internal control of both reactions, two additional primers for amelogenin were used (size at 360 bp) [16]. DNA of mutant homozygote, heterozygote, and wild type samples as controls were run in parallel for each PCR set. Products were separated by horizontal electrophoresis on 2% agarose gel and visualized by staining with ethidium bromide and exposure to ultraviolet light.

Direct sequencing of exon 2 in the GJB2 gene

All the patients found negative or identified in heterozygosity for the c.35delG were further screened by direct sequencing of exon 2 of the *GJB2* gene as previously described [15]. Direct sequencing of the entire coding sequence of the *GJB2* gene was performed using BigDye terminator v1.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA), according to manufacturer's procedure, on an ABI PRISM® 3130XL Genetic Analyzer (Applied Biosystems). Internal primers were also used for the complete cover-up of the coding region of the *GJB2* gene, as described by Neocleous et al. [15].

After receiving results from genetic testing, all families at risk were offered genetic counselling.

RESULTS

Audiological analysis

As described previously, all patients included in this study had ARNSHL which corresponds to autosomal recessive pattern of inheritance. The tympanometry excluded conductive type of deafness in probands. Family history and physical examination excluded the possibility of environmental causes. Audiological analysis performed in the 92 probands demonstrated moderate to profound range of hearing loss (ranging from 41–115 dB) (Figure 1). According to the European Molecular Genetics Quality Network Best Practice Guidelines, patients were classified as having moderate, moderately severe, severe, and profound range of hearing impairment (Table 1) [4]. Mostly they had severe to profound hearing impairment (63/92 patients, 68.48%), while only 6 (6/92, 6.52%) had moderately severe range.

DNA analysis

Ninety-two Serbian patients with autosomal recessive nonsyndromic bilateral sensorineural hearing impairment

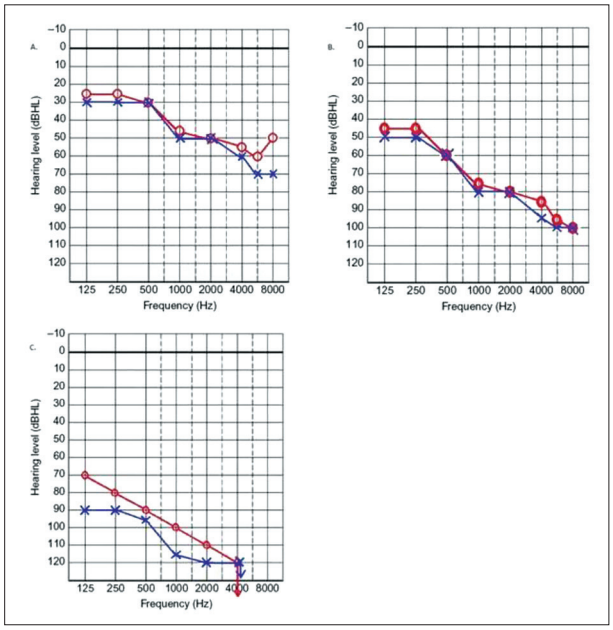

Figure 1. Audiograms in patients with A – moderate, B – severe, C – profound degree of hearing impairment;
o – right ear; × – left ear

Table 1. Results of audiological analysis in a group of 92 Serbian patients with nonsyndromic sensorineural hearing loss

Range of hearing impairment	n (%)
Moderate	23 (25%)
Moderately severe	6 (6.52%)
Severe	29 (31.52%)
Profound	34 (36.96%)
Total	92 (100%)

were initially screened for the presence of c.35delG mutation in the *GJB2* gene. Using the ARMS-PCR method, the c.35delG mutation was detected in 37/92 (40.22%) probands. In this group, c.35delG was nearly equally presented in homozygous and heterozygote state (19/37 and in 18/37 tested patients, respectively) (Figure 2) (Table 2). Further screening of the coding exon 2 was performed in patients found negative or identified in heterozygosity for the c.35delG mutation. In four probands sequencing analysis revealed a second pathogenic variant, while 14 patients had c.35delG in the heterozygous state only (Table 2). In total, all identified pathogenic variants accounted for 42.39% of the mutant alleles (78/184 alleles), while the rest remained uncharacterized. The most frequent mutation was c.35delG mutation and appeared in 56/184 alleles

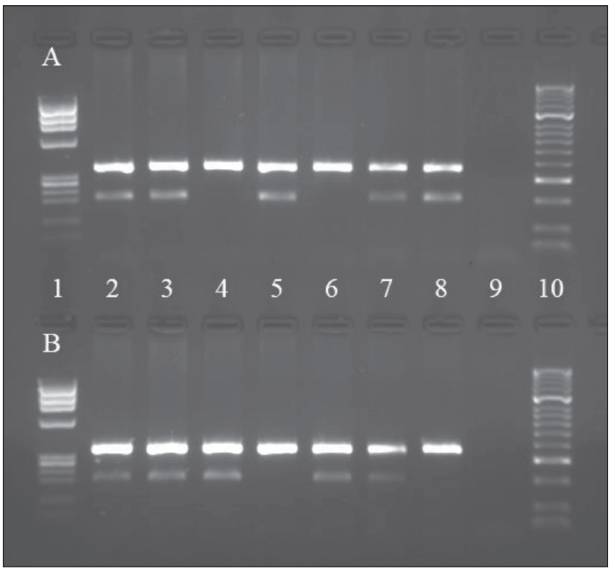

Figure 2. Polymerase chain reaction (PCR) amplification-refractory mutation system products for direct detection c.35delG mutation (202 bp) with internal control (amelogenin, 360 bp), 2% agarose gel, 85V, 1h; A: PCR reaction for detecting normal allele; B: PCR reaction for detecting mutant allele; lane1: GeneRuler 1kb DNA ladder; lanes 2 and 3: samples heterozygous carriers; lane 4: homozygote sample; lane 5: sample wild-type; lane 6: homozygote for the c.35delG control–sample; lane 7: heterozygous carrier control sample; lane 8: wild type control sample; lane 9: blanc; lane 10: ΦX174 DNA-Hae III Digest DNA ladder

(30.43%). To confirm homozygosity and or compound heterozygosity identified in the probands, genetic testing was also done in parents and close relatives (140 relatives in total). Genetic counseling was recommended to all families at risk, considering possibilities of carrier testing and prenatal screening.

Audiologic features in carriers of c.35delG mutation

Thirty-seven of the examined patients were homozygous or heterozygotes for the c.35delG mutation. The majority of these patients were homozygous for the c.35delG mutation (19/37) and had profound degree of hearing impairment (11/19, 57.89%). Four patients (4/37) were found to be compound heterozygotes for the c.35delG mutation and a second pathogenic variant in the *GJB2* gene: c.[35delG];[c.313_326del] in three patients, c.[35delG];[c.71G>A] detected in one patient. All of these patients exhibited profound hearing impairment. The identified patients with only one c.35delG mutation on the one allele and an expected unidentified second mutation

Table 2. Genotype frequencies for the 35delG mutation and association between genotype and degree of hearing impairment in a group of 37 Serbian patients

Genotype	n of probands (%)	Percentage in total cohort of 92 patients	Range of hearing impairment (n of probands)			
			Moderate	Moderately severe	Severe	Profound
c.[35delG];[c.35delG]	19 (51.35%)	20.65	0	2	6	11
c.[35delG];[=]*	14 (37.84%)	15.22	5	0	5	4
c.[35delG];[+]**	4 (10.81%)	4.35	0	0	0	4
Total	37 (100%)	40.22	5	2	11	19

=* – unidentified second pathogenic variant;
+** – identified second pathogenic variant

Table 3. Frequencies of the c.35delG mutation homozygote and compound heterozygote carriers per region

Population	Study	n of probands	c.35delG homozygote carriers (% in total cohort)	c.35delG compound heterozygote carriers (% in total cohort)
Romania	Resmerita et al. [18]	291	58.76	14.4
Hungary	Battellino et al. [24]	194	35.6	12.6
Greece	Battellino et al. [24]	210	30	6.2
Czechia	Battellino et al. [24]	156	28.8	5.1
Croatia	Battellino et al. [24]	63	25.4	7.9
FYR Macedonia	Sukarova Stefanovska et al. [23]	130	25.38	3.85
Slovenia	Battellino et al. [24]	218	21.1	10.5
Serbia	Present study	92	20.65	4.35

(14/37) exhibited moderate to profound hearing loss (Table 2). Finally, our results demonstrated that all carrier patients bearing c.35delG mutation had predominantly severe to profound degree of hearing impairment (30/37, 81.08%).

DISCUSSION

The degree of hearing loss associated with two allele variants in the *GJB2* gene is variable, ranging from mild to profound [8]. A large multicenter genotype–phenotype correlation study by Snoeckx et al. [7] demonstrated that mutations in the *GJB2* gene inactivate the protein product (connexin 26) and cause a more severe phenotype compared to those that do not fully inactivate connexin 26 [8]. Interestingly, this same correlation study also identified variability even among patients with the same genotype. Another study by Cryns et al. [17] also suggested that the severity of hearing impairment is mostly determined by a specific combination of mutations in the *GJB2* gene and that there is no difference in phenotypes in patients with the same or similar genotypes, with different geographical and/or ethnic origin.

Nowadays, c.35delG mutation is widely known as the most prevalent mutation in Caucasians either in the homozygous state or compound heterozygote state in *GJB2* or *GJB6* genes [4, 11]. c.35delG is a truncating, frameshift mutation that leads to premature protein synthesis termination at the 12th amino acid [18]. In the present study, the total frequency of the c.35delG mutant allele was estimated at 30.43% (56/184 alleles) and was identified in 37 patients either in homozygote or heterozygote state (40.22%) (Table 2). The recovered numbers concerning homozygosity and/or heterozygosity of the c35delG are quite similar to the ones previously reported in neighboring populations [10, 18–24] (Table 3).

Nineteen homozygous patients for c.35delG were placed in group 1, since they exhibited severe to profound bilateral sensorineural hearing impairment (Table 2). Among the 19 patients of group 1, 11 exhibited a profound degree of hearing loss with over 100 dB. Only two patients exhibited moderately severe degree of hearing impairment with ~60 dB. Our results are in agreement with many published studies worldwide [18, 19, 25, 26]. In general, the majority of homozygotes for the c.35delG mutation exhibit severe to profound hearing impairment, while few cases develop moderate to even mild hearing impairment [2, 3, 7, 8].

Previously published data showed that there are no significant differences between the audiological parameters in probands who are homozygotes for the c.35delG and compound heterozygous with c.35delG on the one allele [17]. The obtained results of the present study are also in agreement with these findings. In group 2 of the present study there were four patients (4.35% of the total cohort) with the compound heterozygous genotype, all of whom demonstrated profound hearing loss (Table 2). Similar results were found in compound heterozygotes from Romanian and Croatian populations identified in 4.8% and 7.9% of the total cohort, respectively, who mostly exhibited profound degree of hearing impairment [18, 19].

In group 3 of the present study, 14 patients (15.22%) were identified with only one pathogenic mutation and a possible second pathogenic variant remained unidentified due to limitation of the Sanger sequencing methodology. The identification of a possible second pathogenic mutation could be the subject of further analyses using the high-throughput next-generation sequencing technology. The severity of hearing impairment in group 3 ranged from moderate to profound. Therefore, phenotypic variability in group 3 could be further and more precisely explained by the effect of second unidentified disease-causing mutation. The possibility of this phenotypic variability being influenced by modifier gene(s) and/or environmental factors is likable. Previously published data from functional studies suggest that such contribution as a result of modifier genes is considered to be less important [2, 3, 17].

CONCLUSION

The association between genotype and the degree of hearing impairment in patients analyzed in the present study demonstrate that patients with the bi-allelic c.35delG associate with a more severe hearing loss when compared to the ones identified with only one affected allele. Additionally, to some extent, and in agreement with previous studies, the genotype-phenotype correlation in many cases remains controversial and definitely needs further genetic investigation, possibly with the emergence of the next-generation sequencing technology for the identification of other causing genes.

Conflict of interest: None declared.

REFERENCES

1. Kremer H. Hereditary hearing loss; about the known and the unknown. Hear Res. 2019;376:58–68.
2. Sommen M, Wuyts W, Van Camp G. Molecular diagnostics for hereditary hearing loss in children. Expert Rev Mol Diagn. 2017;17(8):751–60.
3. Adadey SM, Wonkam-Tingang E, Twumasi Aboagye E, Nayo-Gyan DW, Boatemaa Ansong M, Quaye O, et al. Connexin Genes Variants Associated with Non-Syndromic Hearing Impairment: A Systematic Review of the Global Burden. Life (Basel). 2020;10(11):258.
4. Hoefsloot LH, Roux AF, Bitner-Glindzicz M; contributors to EMQN DFNB1 best practice meeting. EMQN Best Practice guidelines for diagnostic testing of mutations causing non-syndromic hearing impairment at the DFNB1 locus. Eur J Hum Genet. 2013;21(11):1325–9.
5. Ballana E, Ventayol M, Rabionet R, Gasparini P, Estivill X. Connexins and deafness Homepage [Accessed in March 2020]. Available from: http://www.crg.es/deafness
6. Leiden Open Variation Database. ©2004–2 014 Leiden University Medical Center [Accessed in April 2021]. Available from: https://research.cchmc.org/LOVD2/variants_statistics.php
7. Snoeckx RL, Huygen PL, Feldmann D, Marlin S, Denoyelle F, Waligora J, et al. GJB2 mutations and degree of hearing loss: a multicenter study. Am J Hum Genet. 2005;77(6):945–57.
8. Beach R, Abitbol JM, Allman BL, Esseltine JL, Shao Q, Laird DW. GJB2 Mutations Linked to Hearing Loss Exhibit Differential Trafficking and Functional Defects as Revealed in Cochlear-Relevant Cells. Front Cell Dev Biol. 2020;8:215.
9. Azadegan-Dehkordi F, Ahmadi R, Koohiyan M, Hashemzadeh-Chaleshtori M. Update of spectrum c.35delG and c.-23+1G>A mutations on the GJB2 gene in individuals with autosomal recessive nonsyndromic hearing loss. Ann Hum Genet. 2019;83(1):1–10.
10. Božanić Urbančič N, Battelino S, Tesovnik T, Trebušak Podkrajšek K. The Importance of Early Genetic Diagnostics of Hearing Loss in Children. Medicina (Kaunas). 2020;56(9):471.
11. Koohiyan M. Genetics of Hereditary Hearing Loss in the Middle East: A Systematic Review of the Carrier Frequency of the GJB2 Mutation (35delG). Audiol Neurootol. 2019;24(4):161–5.
12. Jindal S, Chockalingam S, Ghosh SS, Packirisamy G. Connexin and gap junctions: perspectives from biology to nanotechnology based therapeutics. Transl Res. 2021;235:144–67.
13. Srinivas M, Verselis VK, White TW. Human diseases associated with connexin mutations. Biochim Biophys Acta Biomembr. 2017;1860(1):192–201.
14. Scott DA, Kraft ML, Stone EM, Sheffield VC, Smith RJ. Connexin mutations and hearing loss. Nature. 1998;391(6662):32.
15. Neocleous V, Portides G, Anastasiadou V, Phylactou LA. Determination of the carrier frequency of the common GJB2 (connexin-26) 35delG mutation in the Greek Cypriot population. Int J Pediatr Otorhinolaryngol. 2006;70(8):1473–7.
16. Grillo AP, de Oliveira FM, de Carvalho GQ, Medrano RFV, da Silva-Costa SM, Sartorato EL, et al. Single Nucleotide Polymorphisms of the GJB2 and GJB6 Genes Are Associated with Autosomal Recessive Nonsyndromic Hearing Loss. Biomed Res Int. 2015;2015:318727.
17. Cryns K, Orzan E, Murgia A, Huygen PLM, Moreno F, del Castillo I, et al. A genotype–phenotype correlation for GJB2 (connexin26) deafness. J Med Genet. 2004;41(3):147–54.
18. Resmerita I, Cozma RS, Popescu R, Radulescu LM, Panzaru MC, Butnariu LI, et al. Genetics of Hearing Impairment in North-Eastern Romania – A Cost-Effective Improved Diagnosis and Literature Review. Genes. 2020;11(12):1506.
19. Lazăr C, Popp R, Al-Khzouz C, Mihuț G, Grigorescu-Sido P. GJB2 and GJB6 genes mutations in children with non-syndromic hearing loss. Rev Romana de Medicina de Lab. 2017;25(1):37–46.
20. Kecskeméti N, Szönyi M, Gáborján A, Küstel M, Milley GM, Süveges A, et al. Analysis of GJB2 mutations and the clinical manifestation in a large Hungarian cohort. Eur Arch Otorhinolaryngol. 2018;275(10):2441–8.
21. Kokotas H, Grigoriadou M, Hatzaki A, Antoniadi T, Giannoulia-Karantana A, Petersen MB. Easy, rapid, and cost-effective methods for identifying carriers of recurrent GJB2 mutations causing nonsyndromic hearing impairment in the Greek population. Genet Test Mol Biomarkers. 2010;14(2):189–92.
22. Brozkova DF, Marková SP, Mészárosová AU, Jenčík J, Čejnová V, Čada Z, 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. Clin Genet. 2020;98(6):548–54.
23. Sukarova Stefanovska E, Bozhinovski G, Momirovska A, Cakar MD, Sukarova-Angelovska E, Plaseska-Karanfilska D. Mutation analysis of the common deafness genes in patients with nonsyndromic hearing loss in Republic of Macedonia. Mac Med Review. 2017;71(1):20–6.
24. Battellino S, Rudolf G, Zargi M, Trebusak Podkrajsek K, Peterlin B. Connexin 26 (GJB2) and connexin 30 del(GJB6-D13S1830) mutations in Slovenians with prelingual non-syndromic deafness. Int Adv Otol. 2011;7(3):372–8.
25. Azadegan-Dehkordi F, Ahmadi R, Koohiyan M, Hashemzadeh-Chaleshtori M. Update of spectrum c.35delG and c.-23+1G>A mutations on the GJB2 gene in individuals with autosomal recessive nonsyndromic hearing loss. Ann Hum Genet. 2019;83(1):1–10.
26. Mostafa BE, El Sawi MA, Sabry SM, Hassan DM, Rezk Shafik M. Genetic Screening for 35delG Mutation in Egyptian Patients with Profound Sensorineural Hearing Loss Scheduled for Cochlear Implantation: A Population-Based Study. ORL J Otorhinolaryngol Relat Spec. 2021;83(3):181–6.

Аудиолошке карактеристике код болесника из Србије са оштећењем слуха са идентификованом мутацијом *c.35delG* гена *GJB2*

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САЖЕТАК

Увод/Циљ Оштећење слуха представља најчешће сензоринеурално обољење и јавља се са учесталошћу од 1/700–1000 новорођенчади. Варијанте у гену *GJB2*, који кодира протеин конексин 26, најчешћи су узрок аутозомно-рецесивног несиндромског оштећења слуха. Код пацијената са детектованим мутацијама у гену *GJB2* степен оштећења слуха варира од умереног до дубоког.

Циљ овог рада је био утврђивање могућих генотип–фенотип асоцијација између идентификованих генотипова и аудиометријских карактеристика код пацијената из Србије.

Методе Генетичке анализе су спроведене код 92 пацијента помоћу методе *ARMS-PCR* и секвенцирања кодирајућег региона гена *GJB2*. Аудиолошке анализе за процену степена оштећења слуха су урађене код свих пацијената комбинацијом више метода.

Резултати Резултати аудиолошких анализа код 92 пацијента показали су умерени до дубоки степен оштећења слуха. У 42,39% алела идентификоване су патогене варијанте (78/184 алела), а најчешће детектована је *c.*35*delG* варијанта са учесталошћу од 30,43%. Асоцијације генотип–фенотип су испитиване на изолованој групи пацијената (37), који су хомозиготни, сложени хетерозиготни или хетерозиготни носиоци *c.*35*delG* варијанте. У овој групи пацијената најчешћи степен оштећења слуха је тежак до дубок (30/37, 81,08%).

Закључак Код пацијената анализираних у овом раду асоцијација између генотипа и оштећења слуха показује да се код пацијената са биалелским мутацијама (међу којима је *c.*35*delG*) чешће јавља тежи облик оштећења него код пацијената са мутацијом на једном алелу. Већа варирања у степену оштећења слуха код хетерозиготних болесника могу бити објашњена ефектом друге, неидентификоване мутације, генских модификатора или ефектом средине.

Кључне речи: оштећење слуха; ген *GJB2*; варијанта *c.*35*delG*; аудиолошке карактеристике