

GJB2 sequencing in deaf and profound sensorineural hearing loss children

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ABSTRACT:

Introduction. GJB2 mutations are the most frequent reason of genetic congenital hearing loss. The aim of the study was to assess the prevalence of GJB2 mutations in the deaf and profound hearing loss children.

Material and methods. The material of the study was a group of 61 patients divided into two groups. Group I – 35 deaf or with profound sensorineural hearing loss children (the pupils of the deaf and hard of hearing school), aged 5–17 years (average 9,2 years), 14 males, 21 females, II – control group comprised 26 normal hearing patients, aged 5–16 years (average 10,4 years), 14 males, 12 females (patients of Department of Pediatric Otolaryngology, Audiology and Phoniatics, Medical University of Lodz). In both groups, exon 2 sequencing of GJB2 gene was performed.

Results. In group I, in 6 patients (17%) 35delG in GJB2 gene was found. The patients were homozygotes, with negative family history of hearing loss. No other mutations in GJB2 gene were found. In group II no mutations in GJB2 were observed.

Conclusions. The most frequent cause of hearing impairment in the deaf and profound sensorineural hearing loss children was 35delG mutation in GJB2 gene. No other mutations in GJB2 gene were detected.

KEYWORDS:

deafness, connexin 26, GJB2 sequencing

INTRODUCTION

Sensorineural hearing loss is the most common deficit of the sensory system. It is estimated that deafness or profound hearing loss can be observed in 1–3 per 1000 births. Approximately 50% of cases of prelingual hearing loss has a genetic background.

The GJB2 gene, encoding connexin 26, located on chromosome 13q11 was the first identified gene responsible for isolated, autosomal recessive hearing loss (DFNB1). Mutations in this gene are the most common cause of congenital hearing loss with a genetic background. Other cases in this group of deafness include for example environmental factors such as viral, bacterial infections, ototoxic substances and traumas, including acoustic ones.

The DFNB1 locus was described for the first time in 1994, and first mutations in this locus were observed in 1997. [1,2]. This

locus contains two genes – GJB2 and GJB6, encoding connexin 26 and connexin 30, respectively. Connexins are gap junction components, they are present in the cell membranes of the epithelial cells and connective tissue of the cochlea, and are responsible for maintaining an electric potential in the cochlea. Gap junctions are used to exchange neurotransmitters, metabolites and to transport potassium ions.

In a typical situation, GJB2 mutations are inherited in an autosomal recessive pattern; however, there have been reported forms with a dominant pattern of inheritance (DFNA3), non-syndromic [3,4] or associated with skin lesions in the course of genetic conditions combined with deafness [5,6].

Pampanos et al. report pseudodominant inheritance of deafness in relation to a high rate of homozygotes for the 35delG mutation [7]. Including polymorphisms, there are known more than 300 mutations in the GJB2 gene, leading to perceptible hearing

loss [8]. Some of them are observed more often in various populations, for example the 35delG mutation (guanine deletion at position 35) in the Caucasian population [9,10], 235delC in the Asian population [11], 167delT in the Jewish population [12,13], p. Trp24 in the population of India, Bangladesh, Slovenia and in the Romani people [14–17].

The paper aimed to assess the incidence of mutations in the connexin 26 gene in a group of deaf children or children with profound sensorineural hearing loss.

MATERIAL AND METHODS

Tests were performed in 61 patients who were divided into two groups: 1 – study group – 35 children with deafness or significant perceptive hearing loss (pupils of the Deaf and Hard of Hearing School No. 4 in Łódź), aged 5–17 years (mean 9.2 years), including 14 boys and 21 girls, 2 – control group – 26 patients with normal hearing, aged 5–16 years (mean 10.4 years), including 14 boys and 12 girls (patients of the Pediatric Department of Otolaryngology, Audiology and Phoniatrics, Medical University of Lodz).

Prior to the study, children's legal guardians filled in a questionnaire with questions regarding hearing loss in a given child and in their family, as well as any risk factors for hearing loss in the past. When a legal guardian signed an informed consent form regarding participation in the study approximately 3 mL of blood were collected into a tube with EDTA. DNA was isolated from 200 µL of blood using the GeneMATRIX Quick Blood DNA Purification Kit (EurX), according to the manufacturer's guidelines. The levels and quality of DNA obtained were assessed spectrophotometrically (Picodrop). The exon 2 of the GJB2 gene was sequenced. The analysis was performed in a cycler using a set of reagents, BigDye® Terminator v1.1 by Applied Biosystems (Life Technologies). Products of a sequencing reaction were separated in a capillary sequencer 3130xl Genetic Analyzer. Starters used for the reaction of GJB2 sequencing based on Denoyelle et al. (1997):

GJB2F 5'-TCTTTTCCAGAGCAAACCGCC-3'; GJB2R 5'-TGAGCACGGTTGCCTCATC-3';

GJB2iF: 5'-GACACGAAGATCAGCTGCAG-3'; GJB2iR 5'-CCAGGCTGCAAGAACGTGTG-3'.

Sequencing conditions according to a protocol by Applied Biosystems – GeneAmp PCR System 9700 (initial denaturation – 96°C – 1 minute, 25 cycles: 96°C – 10 seconds, 50°C – 5 seconds, 60°C – 4 minutes).

The Bioethics Committee at the Medical University of Lodz approved the study (RNN/292/15/KE).

STUDY RESULTS

As a result of sequencing of exon 2 of the GJB2 gene, a mutation 35delG was observed in 6 cases in group 1 (17%). Cases included homozygous patients from families with normal hearing. In this group no other mutations were detected.

Sequencing excluded mutations of exon 2 of the GJB2 gene in group 2 (control group).

In group 1, a risk factor for hearing loss was observed in 6 subjects (17%). In 4 cases (11%) these factors included treatment with aminoglycosides (it coexisted, as a risk factor for hearing loss, with the 35delG mutation in 2 cases), in 1 case (3%) – low birth weight below 1500 mg, and in 1 case (3%) – head trauma.

In group 2 (control group) there were 2 cases where a risk factor for hearing loss was identified – in 1 case (4%) a child had had a head trauma with loss of consciousness, and in one case (4%) a mother had had cytomegalovirus infection during pregnancy.

DISCUSSION

A group of patients with hearing loss with a genetic background is extremely heterogeneous. More than 150 loci and more than 100 genes are known that are responsible for such types of hearing loss. Genetically conditioned prelingual deafness is usually associated with a mutation in the connexin 26 gene. The majority of such hearing loss cases are detected during hearing screening tests in newborns; however, there are rare cases of delayed or progressive sensorineural hearing loss [18].

Norris et al. [19] reported cases of delayed deafness in children with normal results of hearing screening tests in newborns in whom a mutation in the GJB2 gene was later found.

The incidence of mutations in the connexin 26 gene in Europe varies. According to the literature, it is the highest in the south, in the Mediterranean countries, and the lowest in the north [20,21]. Polish studies report that the reason for congenital deafness is genetically conditioned deafness (associated with the GJB2 mutation) in 40% of cases [22,23].

In the study group the result was lower (17%), and it may be associated with a low study sample or imprecise genetic testing. Due to a relatively high incidence of mutations in the

GJB2 and GJB6 genes in the European population and in the USA (a few percent of the whole population) some countries even perform screening tests for this abnormality [9, 19, 24].

The European Molecular Genetics Quality Network presents their recommendations on the diagnostic tests for genetically conditioned hearing loss [25]. However, this report does not recommend tests for single point mutations, such as 35delG. The authors indicate that screening tests for the most common genetic abnormalities are necessary, and in case of negative results they recommend sequencing of exon 2, analysis of 5' and 3' ends of intron 1 and search for deletions in GJB6. Additionally, if at least one point mutation in the GJB2 gene has been diagnosed it is recommended to search for deletions in GJB6.

The 35delG mutation results in the reading frame shift, namely early completion of protein translation. Consequently, patients homozygous for this mutation demonstrate hearing loss (mild to profound) whereas heterozygotes may have normal hearing [26, 27].

Burke et al. and Kim et al. demonstrated an increased rate of heterozygotes (monoallelic mutations) in a group with sensorineural hearing loss compared to a control group with normal hearing [28,29].

In mice, reduced expression of connexin 26 18 days after birth resulted in an increased susceptibility of the normal organ of Corti to acoustic traumas and degenerative lesions [30]. Studies by Van Eyken et al. in human patients indicate a lack of a correlation between the GJB2 mutations and increased susceptibility to acoustic traumas or ageing of the auditory system [31]. However, A1555G mutations of the 12s rRNA mitochondrial subunit demonstrate such a correlation as they increase the susceptibility to ototoxic injuries caused by aminoglycosides [32].

Within recent years the availability and popularity of genetic screening tests for congenital hearing loss have increased. Screening tests for the most common mutations in the GJB2, GJB6, SLC26A4, and OTOF genes are performed [33]. Consequences of detailed diagnostic genetic tests include more extensive genetic counselling in order to predict future hearing loss in the offspring. This, on the other hand, results in the use of various therapeutic options (early use of hearing aids, cochlear implants) to create conditions for normal development of speech. Diagnostic genetic tests for hearing loss associated with the GJB2 mutation are also used in prenatal tests and in preimplantation tests performed prior to *in vitro* fertilisation [34, 35].

Thanks to genetic engineering it is possible to start genetic therapy in cases of genetically conditioned hearing loss (for example, viral vectors replacing copies of a damaged gene in the inner ear). In animal studies improved hearing was observed after administration of adenovirus with the SLC17A8 gene into the cochlea in mice. In other studies the administration of the ATOH1 gene (a gene encoding one of transcription factors) in mice made it possible for the auditory cells in the cochlea to develop and regenerate [36, 37].

Despite great opportunities made available by genetic therapy, Cooke-Hubley and Middleton draw attention to negative attitudes of the population of the deaf to diagnostic genetic tests for hearing loss and genetic counselling [38].

CONCLUSIONS

The 35delG mutation of the GJB2 gene was the most common cause of genetically conditioned hearing loss in the population of deaf children and children with profound sensorineural hearing loss.

In the study population no other mutations in the GJB2 gene were detected.

REFERENCES

1. Guilford P, Ben Arab S, Blanchard S, Levilliers J, Weissenbach J, Belkahia A. et al.: A non-syndrome form of neurosensory, recessive deafness maps to the pericentromeric region of chromosome 13q. *Nat. Genet.* 1994; 6 (1): 24–28.
2. Kelsell D.P., Dunlop J., Stevens H.P., Lench N.J., Liang J.N., Parry G. et al.: Connexin 26 mutations in hereditary non-syndromic sensorineural deafness. *Nature*, 1997; 387 (6628): 80–83.
3. Bazazzadegan N, Sheffield A.M., Sobhani M., Kahrizi K., Meyer N.C., Van Camp G. et al: Two Iranian families with a novel mutation in *GJB2* causing autosomal dominant nonsyndromic hearing loss. *Am. J. Med. Genet. A.* 2011; 155A:1202–1211.
4. Denoyelle F, Lina-Granade G, Plauchu H, Bruzzone R, Chaïb H, Lévi-Acobas F. et al.: Connexin 26 gene linked to a dominant deafness. *Nature*, 1998; 393: 319–320.
5. Mazereeuw-Hautier J, Bitoun E., Chevrant-Breton J., Man S.Y., Bodemer C., Prins C. et al: Keratitis-ichthyosis-deafness syndrome: disease expression and spectrum of connexin 26 (*GJB2*) mutations in 14 patients. *Br. J. Dermatol.* 2007; 156: 1015–1019.
6. De Zwart-Storm E.A., Hamm H., Stoevesandt J., Steijlen P.M., Martin P.E., van Geel M. et al.: A novel missense mutation in *GJB2* disturbs gap junction protein transport and causes focal palmoplantar keratoderma with deafness. *J. Med. Genet.* 2008; 45: 161–166.



7. Pampanos A., Neou P., Iliades T., Apostolopoulos N., Voyiatzis N., Grigoriadou M. et al.: Pseudodominant inheritance of DFNB1 deafness due to the common 35delG mutation. *Clin. Genet.* 2000; 57 (3): 232–234.
8. Stenson P.D., Mort M., Ball E.V., Shaw K., Phillips A.D., Cooper D.N.: The human gene mutation database: building a comprehensive mutation repository for clinical and molecular genetics, diagnostic testing and personalized genomic medicine. *Hum. Genet.* 2014; 133 (1): 1–9.
9. Gasparini P., Rabionet R., Barbujani G., Melchionda S., Petersen M., Brøndum-Nielsen K. et al.: High carrier frequency of the 35delG deafness mutation in European populations. Genetic Analysis Consortium of *GJB2* 35delG. *Eur. J. Hum. Genet.* 2000; 8: 19–23.
10. Lucotte G., Mercier G.: Meta-analysis of *GJB2* mutation 35delG frequencies in Europe. *Genet. Test.* 2001; 5: 149–152.
11. Yan D., Park H.J., Ouyang X.M., Pandya A., Doi K., Erdenetungalag R. et al.: Evidence of a founder effect for the 235delC mutation of *GJB2* (connexin 26) in east Asians. *Hum. Genet.* 2003; 114: 44–50.
12. Marlin S., Feldmann D., Blons H., Loundon N., Rouillon I., Albert S. et al.: *GJB2* and *GJB6* mutations: genotypic and phenotypic correlations in a large cohort of hearing-impaired patients. *Arch. Otolaryngol. Head Neck Surg.* 2005; 131 (6): 481–487.
13. Sobe T., Vreugde S., Shahin H., Berlin M., Davis N., Kanaan M. et al.: The prevalence and expression of inherited connexin 26 mutations associated with non-syndromic hearing loss in the Israeli population. *Hum. Genet.* 2000; 106 (1): 50–57.
14. Alvarez A., del Castillo I., Villamar M., Aguirre L.A., González-Neira A., López-Nevot A., et al.: High prevalence of the W24X mutation in the gene encoding connexin-26 (*GJB2*) in Spanish Romani (gypsies) with autosomal recessive non-syndromic hearing loss. *Am. J. Med. Genet. A.* 2005; 137A: 255–258.
15. Joseph A.Y., Rasool T.J.: High frequency of connexin26 (*GJB2*) mutations associated with nonsyndromic hearing loss in the population of Kerala, India. *Int. J. Pediatr. Otorhinolaryngol.* 2009; 73: 437–443.
16. Minarik G., Ferak V., Ferakova E., Ficek A., Polakova H., Kadasi L.: High frequency of *GJB2* mutation W24X among Slovak Romany (Gypsy) patients with non-syndromic hearing loss (NSHL). *Gen. Physiol. Biophys.* 2003; 22: 549–556.
17. Padma G., Ramchander P.V., Nandur U.V., Padma T.: *GJB2* and *GJB6* gene mutations found in Indian probands with congenital hearing impairment. *J. Genet.* 2009; 88: 267–272.
18. Pagarkar W., Bitner-Glindzicz M., Knight J., Sirimanna T.: Late postnatal onset of hearing loss due to *GJB2* mutations. *Int. J. Pediatr. Otorhinolaryngol.* 2006; 70 (6): 1119–1124.
19. Norris V.W., Arnos K.S., Hanks W.D., Xia X., Nance W.E., Pandya A.: Does universal newborn hearing screening identify all children with *GJB2* (Connexin 26) deafness? Penetrance of *GJB2* deafness. *Ear Hear.* 2006; 27 (6): 732–741.
20. Amorini M., Romeo P., Bruno R., Galletti F., Di Bella C., Longo P. et al.: Prevalence of Deafness-Associated Connexin-26 (*GJB2*) and Connexin-30 (*GJB6*) Pathogenic Alleles in a Large Patient Cohort from Eastern Sicily. *Ann. Hum. Genet.* 2015 Jun 19.
21. Siem G., Fagerheim T., Jonsrud C., Laurent C., Teig E., Harris S. et al.: Causes of hearing impairment in the Norwegian paediatric cochlear implant program. *Int. J. Audiol.* 2010; 49 (8): 596–605.
22. Śliwińska-Kowalska M., Kotyło P., Borowiec M., GajdaSzadkowska A., Kowalski M.L.: Występowanie mutacji 35delG w genie koneksyny 26 wśród dzieci głuchych i niedosłyszących oraz ich rodziców. *Otorynolaryngologia*, 2003; 2 (3): 126–132.
23. Wiszniewski W., Sobieszczńska-Radoszewska L., Nowakowska-Szyrwińska E., Obersztyn E., Bal J.: High frequency of *GJB2* gene mutations in Polish patients with prelingual nonsyndromic deafness. *Genet. Test.* 2001; 5 (2): 147–148.
24. Frei K., Ramsebner R., Lucas T., Baumgartner W.D., Schoefer C., Wachtler F.J. et al.: Screening for monogenetic del(*GJB6*-D13S1830) and digenic del(*GJB6*-D13S1830)/*GJB2* patterns of inheritance in deaf individuals from Eastern Austria. *Hear Res.* 2004; 196 (1–2): 115–118.
25. Hoefsloot L.H., Roux A.F., 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): 1325–1329.
26. Cohn E.S., Kelley P.M., Fowler T.W., Gorga M.P., Lefkowitz D.M., Kuehn H.J. et al.: Clinical studies of families with hearing loss attributable to mutations in the connexin 26 gene (*GJB2*/DFNB1) *Pediatrics.* 1999; 103: 546–550.
27. Murgia A., Orzan E., Polli R., Martella M., Vinanzi C., Leonardi E. et al.: Cx26 deafness: Mutation analysis and clinical variability. *J. Med. Genet.* 1999; 36: 829–832.
28. Burke W.F., Warnecke A., Schöner-Heinisch A., Lesinski-Schiedat A., Maier H., Lenarz T.: Prevalence and audiological profiles of *GJB2* mutations in a large collective of hearing impaired patients. *Hear Res.* 2016; 333: 77–86.
29. Kim S.Y., Kim A.R., Kim N.K., Lee C., Kim M.Y., Jeon E.H. et al.: Unraveling of Enigmatic Hearing-Impaired *GJB2* Single Heterozygotes by Massive Parallel Sequencing: DFNB1 or Not? *Medicine (Baltimore)*. 2016; 95 (14): 3029.
30. Zhou X.X., Chen S., Xie L., Ji Y.Z., Wu X., Wang W.W. et al.: Reduced Connexin26 in the Mature Cochlea Increases Susceptibility to Noise-Induced Hearing Loss in Mice. *Int. J. Mol. Sci.* 2016; 26: 17 (3). pii: E301.
31. Van Eyken E., Van Laer L., Franssen E., Topsakal V., Hendrickx J.J., Demeester K. et al.: The contribution of *GJB2* (Connexin 26) 35delG to age-related hearing impairment and noise-induced hearing loss. *Otol. Neurotol.* 2007; 28 (7): 970–975.
32. Prezant T.R., Agopian J.V., Bohlman M.C., Bu X., Oztas S., Qiu W.Q. et al.: Mitochondrial ribosomal RNA mutation associated with both antibiotic-induced and non-syndromic deafness. *Nat. Genet.* 1993; 4 (3): 289–294.
33. Hilgert N., Smith R.J., Van Camp G.: Forty-six genes causing nonsyndromic hearing impairment: which ones should be analyzed in DNA diagnostics? *Mutat. Res.* 2009; 681 (2–3): 189–196.
34. Coviello D.A., Brambati B., Tului L., Percesepe A., Sironi F., Sahai A. et al.: First-trimester prenatal screening for the common 35delG *GJB2* mutation causing prelingual deafness. *Prenat. Diagn.* 2004 Aug; 24 (8): 631–634.

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35. Liss J., Mirecka A., Kitowska K., Lukaszuk K.: Preimplantation genetic diagnosis of hearing loss with 35delG mutation in *GJB2* gene – preliminary report. *Otolaryngol. Pol.* 2011; 65 (6): 443–446.
 36. Akil O., Seal R.P., Burke K., Wang C., Alemi A., Daring M. et al.: Restoration of hearing in the VGLUT3 knockout mouse using virally mediated gene therapy. *Neuron* 2012; 75 (2): 283–293.
 37. Lustig L.R., Akil O.: Cochlear gene therapy. *Current Opinion in Neurology.* 2012; 25 (1): 57–60.
 38. Middleton A., Emery S.D., Turner G.H.: Views, knowledge and beliefs about genetics and genetic counseling among deaf people. *Sign. Lang. Stud.* 2010; 10 (2): 170–196.
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