International Journal of Pediatric Otorhinolaryngology 76 (2012) 90–94

Contents lists available at SciVerse ScienceDirect

International Journal of Pediatric Otorhinolaryngology

journal homepage: www.elsevier.com/locate/ijporl

Prevalence of mutations located at the *dfnb1* locus in a population of cochlear implanted children in eastern Romania

Luminiţa Rădulescu [a,b,*], Cristian Mârţu [a,b], Ralf Birkenhäger [c], Sebastian Cozma [a,b], Loreta Ungureanu [a,b], Roland Laszig [c]

[a] University of Medicine and Pharmacy "Gr. T. Popa", Iaşi, Romania
[b] Department of Otorhinolaryngology, Head and Neck Surgery, Clinical Rehabilitation Hospital, Iaşi, Romania
[c] Department of Otorhinolaryngology, Head and Neck Surgery, University Medical Center Freiburg, Germany

ARTICLE INFO

Article history:
Received 8 August 2011
Received in revised form 5 October 2011
Accepted 7 October 2011
Available online 8 November 2011

Keywords:
Genetic hearing loss
GJB2
DFNB1
Connexin
Cochlear implant

ABSTRACT

Objective: Hearing loss is one of the major public health problems, with a genetic etiology in more than 60% of cases. Connexin 26 and connexin 30 mutations are the most prevalent causes of deafness. The aim of this study is to characterize and to establish the prevalence of the *GJB2* and *GJB6* gene mutations in a population of cochlear implanted recipients from Eastern Romania, this being the first report of this type in our country.

Methods: We present a retrospective study that enrolled 45 Caucasian cochlear implanted patients with non-syndromic sensorineural severe to profound, congenital or progressive with early-onset idiopathic hearing loss. We performed sequential analysis of exon 1 and the coding exon 2 of the *GJB2* gene including also the splice sites and analysis of the deletions del(GJB6-D13S1830), del(GJB6-D13S1854) and del(chr13:19,837,343–19,968,698).

Results: The genetic analysis of the *GJB2* gene identified connexin 26 mutations in 22 patients out of 45 (12 homozygous for c.35delG, 6 compound heterozygous and 4 with mutations only on one allele). We found 6 different mutations, the most prevalent being c.35delG – found on 32 alleles, followed by p.W24* – found on 2 alleles. We did not identify the deletions del(GJB6-D13S1830), del(GJB6-D13S1854) and del(chr13:19,837,343–19,968,698).

Conclusions: Although the most prevalent mutation was c.35delG (80% from all types of mutations), unexpectedly we identified 5 more different mutations. The presence of 6 different mutations on the GJB2 gene has implications in hearing screening programs development in our region and in genetic counseling.

1. Introduction

Hearing loss (HL) is the most common sensory disorder in human, with an incidence at birth of 1 to 650 newborns [1,2] and a prevalence in the population of 10–12% [3,4].

Due to its high prevalence, HL is placed amongst the major public health problems, hence the need of screening programs development [5,6]. The data collected from the universal newborn hearing screening, which we perform in our region, shows that out of the 7077 tested newborns in 2008 by otoacoustic emissions (OAE) – distortion products – 8 children were confirmed with bilateral severe or profound sensorineural hearing loss (SNHL), meaning 1.13 in 1000 newborns for our region.

The main purpose for early detection of children with HL is conventional hearing aid fitting or cochlear implantation at the right moment for optimal auditory and speech rehabilitation of the child [7].

As known, rehabilitation results are mainly related to the cochlear implantation age [8,9], but also to other factors among which probably the HL etiology [10]. HL can have a genetic cause, can be acquired or can be multifactorial [11].

Genetic deafness can be syndromic in 30% of cases (when it is associated with other pathologic findings) or nonsyndromic in 70–80% of the total genetic deafness [12] – when it appears isolated to a seemingly normal person.

Genetic mutations responsible for HL cases can occur in autosomal or gonosomal chromosomes but also in mitochondrial DNA [11,13,14].

The autosomal recessive forms represent about 80% of the total nonsyndromic deafness [15], usually characterized by a sensorineural type involving the whole frequency range [16]. Most of these cases are severe forms with prelingual onset although some

* Corresponding author at: Prof. A. Sesan Street, No. 6, Iaşi 700046, Romania. Tel.: +40 723 798182; fax: +40 232 222842.
E-mail addresses: lmradulescu@yahoo.com, radulesculuminita@ymail.com (L. Rădulescu).

0165-5876/$ – see front matter © 2011 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.ijporl.2011.10.007

cases of postlingual onset with progressive evolution have also been described [17]. The *DFNB1* locus on chromosome 13q11–12 is the most frequently affected one. Mutations located at this level are responsible for more than 50% of the autosomal recessive sensorineural hearing loss (ARSNHL) [18]. Two genes have been associated with the *DFNB1* locus – *GJB2* and *GJB6* – involved in the auditory function by encoding the gap-junction proteins connexin 26 and connexin 30 [19,20]. Both connexins are components of the gap-junction channels that mediate electrolytic and other metabolites changes required for the inner ear function [21].

In the *GJB2* gene there were 91 described mutations, out of which 89 with recessive features. The prevalence of each mutation is different from one population to another according to the founder effect theory [22,23]. In the Caucasian population the most frequent mutation is c.35delG (approximately 2/3 of cases) [23,24]. In the Asian population the c.235delC mutation is the most encountered [25–27], while in the Jewish population the most frequent mutation is c.167delT [28]. The p.R143W mutation is predominant among certain Africans [29] and the p.W24* mutation among European Gypsy and Indian people [30].

Mutations responsible for deafness can be monogenic (only one gene involved) or digenic (simultaneous involvement of 2 genes). Related to this, the role of *GJB6* gene adjacent to *GJB2* gene on the *DFNB1* locus is described [31]. The most frequent mutation in the *GJB6* gene is a deletion – the 309-kb deletion [32]. This deletion induces deafness either in homozygote form or in compound heterozygous form, in the last case only if there is a mutation at the level of the *GJB2* gene on the pair chromosome. The prevalence of this deletion in the *GJB6* gene is variable depending on the studied population [33].

Aim. All around the world significant efforts are being made to achieve the descriptive epidemiology related to connexin 26 mutations; in this regard, the main purpose of this study is to characterize and establish the prevalence of mutations at the level of the *GJB2* gene, and to evaluate the prevalence of the deletions del(*GJB6*-D13S1830), del(*GJB6*-D13S1854) and del(chr13:19,837,343–19,968,698) in the *GJB6* gene in a population of cochlear implanted recipients in Eastern Romania, which makes it the first study of this type in our country.

The related objectives are: to contribute to the existing international database by gathering the information necessary to establish the implications of connexin 26 mutations in auditory and speech rehabilitation in cochlear implanted recipients and to gather useful data necessary for the genetic counseling and for the genetic screening of deafness.

2. Materials and methods

2.1. Patients

The study was performed on 45 patients from Eastern Romania, 25 males and 20 females (unrelated to each other) evaluated, diagnosed and cochlear implanted in the Department of Otorhinolaryngology, Head and Neck Surgery, Clinical Rehabilitation Hospital, University of Medicine and Pharmacy "Gr. T. Popa" in Iasi, Romania. The Ethics Committee of the University of Freiburg approved this part of the project (no. 161/02-07/2003/Birkenhäger). The study was also approved by the Ethics Committee of the Clinical Rehabilitation Hospital Iasi (no. 12511 – 10.07.2009).

The evaluation protocol consisted of: medical history of the disease, especially regarding the onset modality and evolution of deafness, associated symptoms and their development, past personal and family medical history to exclude any possible family interrelations and other possible causes of deafness, complete ENT examination – especially otomicroscopic exam, subjective and objective audiologic tests (pure tone and speech audiometry, OAE, auditory steady state response, tympanogram, auditory brainstem responses), vestibular testing, speech therapist examination, imaging testing – CT and MRI. Also, interdisciplinary complex examination (ophthalmologic, pediatric, clinical genetic, psychiatric, etc.) was performed to exclude syndromic forms.

All patients included in this study were cochlear implant users for congenital nonsyndromic or early onset idiopathic progressive severe (71–90 dB) to profound (>90 dB) SNHL. All of them have worn conventional hearing aids for at least 6 months – without any benefits – before being implanted. All were unilaterally implanted when included in the study. In cases with asymmetrical HL, the most impaired ear was implanted.

2.2. Molecular genetic analysis

Genomic DNA of patients was extracted from peripheral blood leukocytes of the patients using the standard methods (Qiagen, Hilden Germany). Primer and PCR conditions were selected according to procedures optimized previously for sequence analysis of exon 1 and the coding exon 2 of the *GJB2* gene, including all splice sites [34] and analysis of the *GJB6* deletions del(*GJB6*-D13S1830), del(*GJB6*-D13S1854) and del(chr13:19,837,343–19,968,698) [35]. Sequencing of the PCR products was done with standard procedures and analyzed in an automated DNA sequencer Amersham MegaBACE™ 500 (Amersham Biosciences, GE Healthcare Europe, München, Germany).

For the examination of the *GJB6* gene (connexin 30) deletions the breakpoint junctions of del(*GJB6*-D13S1830) and del(*GJB6*-D13S1854) were analyzed according to del Castillo et al. (2002, 2005) [31,32], the deletion del(chr13:19,837,343–19,968,698) was analyzed according to Wilch et al. (2010) [35].

Informed consent was obtained from the patients, parents or legal guardians for children before collecting blood for genetic testing.

3. Results

Our study included 45 cochlear implanted patients, 43 had bilateral congenital severe to profound SNHL and 2 had progressive HL that required cochlear implantation at ages of 6 and 8 years, respectively. The genetic analysis of the *GJB2* (connexin 26) and *GJB6* (connexin 30) genes identified in 22 (48.8%) patients mutations in the *GJB2* gene: 12 (26.6%) patients were homozygous for the c.35delG mutation; 5 (11.1%) patients were compound heterozygous with c.35delG mutation on one allele and a different mutation on the other allele; 1 (2.2%) patient was compound heterozygous for two different mutations non c.35delG; in 4 (8.8%) patients only one mutated allele was identified (Table 1).

The analysis of 90 alleles revealed 6 different mutations, four of these alterations are truncating mutations, c.35delG, c.71G>A

Table 1
GJB2 and *GJB6* genotypes found in 22 subjects out of a collective of 45 patients with non-syndromic hearing loss.

Compound homozygous	
c.35delG/c.35delG	12
Compound heterozygous	
c.35delG/c.313_326del14	2
c.35delG/c.71G>A	1
c.35delG/c.551G>C	2
c.71G>A/c.299_300delAT	1
Heterozygous affected	
wt/c.35delG	3
wt/c.358_360delGAG	1
Total mutated subjects	22
No mutation found	23
Total subjects	45

Table 2
Spectrum of *GJB2* (Connexin 26) mutations detected in non-syndromic hearing loss in our group.

Nucleotide	Protein	Classification	Mutation type	No.	References
c.35delG	p.Gly12Valfs*2	T	Deletion/Nonsense	32	Denoyelle et al. (1997) [48]
c.71G>A.	p.Trp24* (p.W24*)	T	Nonsense	2	Kelsell et al. (1997) [49]
c.299_300delAT		T	Frameshift	1	Abe et al. (2000) [26]
c.313_326del14	p.Lys105Glyfs*5	T	Deletion/Frameshift	2	Denoyelle et al. (1999) [50]
c.358_360delGAG	p.delGlu120 (del E120)	NT	In Frame Deletion	1	Denoyelle et al. (1999) [50]
c.551G>C	p.Arg184Pro (p.R184P)	NT	Missense	2	Denoyelle et al. (1997) [48]
T, Truncating mutation; NT, non-truncating mutation			Total mutated alleles:	40	

p.W24*, c.299_300delAT and c.313_326del14 (AAGTTCATCAAGGG), the two other alterations are non-truncating mutations, c.358_360delGAG p.delE120 is a in frame deletion and c.551G>C p.R184P is a missense mutation. The truncating mutation c.35delG represents 80% of all the mutated *GJB2* (connexin 26) alleles, the prevalence of this mutation is 35.5% (32/90) in the investigated collective of patients all the mutations p.W24*, p.R184P and c.313_326del14 have a prevalence of 2.2% (2/90) and the mutations c.299_300delAT, and p.del E120 have a prevalence of 1.1% (1/90) (Table 2).

The *GJB6* (connexin 30) deletions *GJB6*-D13S1830, *GJB6*-D13S1854 and del(chr13:19,837,343–19,968,698) were not detected in our patients (Lerer et al., 2001 [36], del Castillo 2002, 2005 [31,32], Wilch et al., 2010 [35]).

Additionally no polymorphisms were identified.

Additionally exon 1 was analyzed in all individuals who proved to be heterozygous for only one coding mutation, to identify the splice-site mutation IVS1+1G>A.

Both patients with progressive SNHL were compound heterozygous (c.35delG + c.313_ 326del14 and c.35delG + c.71G>A p.W24*).

4. Discussion

The present study, like many others published in the recent years, describes the genetic mutations present on the *DFNB1* locus in cochlear implanted recipients. Although, significant efforts have been made worldwide to define the epidemiology of *GJB2* and *GJB6* genes mutations related to HL, the issue remains open for some populations. This is the first report in Romania of the genetic profile of a group of cochlear implanted recipients with bilateral severe to profound SNHL due to mutations in the *GJB2* and *GJB6* genes.

A previous study, performed in the Northwest region of Romania, determined only the prevalence of two mutations (c.35delG and p.W24*) in the *GJB2* gene, in non-cochlear implanted patients with different degrees of SNHL [34,37].

As our study enrolled only patients with idiopathic deafness, a large number of cases (40%) with genetic HL secondary to *DFNB1* mutations were identified, which is consistent with the literature data according to which the *GJB2* mutations are the most frequent cause of ARSNHL in most world populations, accounting for up to 50% of ARSNHL cases [16]. These findings emphasize the importance of GJB2 screening in our population for early detection of severe to profound hearing loss.

The c.35delG mutation is most frequently occurring in the Caucasian populations and may account for up to 70% of all *GJB2* mutations. As we had expected the most frequent mutation in our group was c.35delG in homozygote state present in 66.7% of the patients with *GJB2* mutations.

The high number of mutations identified in the *GJB2* gene (6 different mutations in 22 of the 45 tested patients – Table 1) in our patients has at least two implications:

(1) It points out the high prevalence of GJB2 mutations in cochlear implanted children from our region (when compared with studies that had found low incidence of GJB2 mutations in certain populations with severe to profound SNHL) demonstrating the importance of GJB2 analysis in our population.
(2) The presence of 5 mutations, different from c.35delG, in 33.3% of cases emphasizes the importance of genetic analysis by direct sequencing of the entire *GJB2* gene rather than only by genetic testing common mutations exclusively [38]; according to the connexin-deafness homepage, 91 different mutations have been identified, some of which are very frequent and others are extremely rare. These mutations occur with different frequency across populations. As the mutations reported after 2003 are not listed, an extensive literature search completed by Hilgert et al. estimates that over 220 mutations have been reported. In the patients here under analysis no new mutations had been observed [20].

The second most common mutation (p.W24*) was found in compound heterozygous form in 2 of 18 patients with connexin 26 mutation on both alleles (Table 1). This mutation is considered characteristic for the European Gypsy and Indian Population [30]. These findings are similar with those reported by other authors for the Central and Eastern Europe [31,39–42].

Four (8.9%) heterozygous patients were found, 3 (6.66%) for the c.35delG mutation and 1 (2.22%) for the c.358_360delGAG without any other alteration either on the *GJB2* or *GJB6* gene on the second allele. This cannot explain the hearing impairment of these patients [25]. The etiology of deafness in these patients is possible to be secondary either to a nongenetic factor or to another mutation unrelated to *DFNB1* locus and the affected patients can be carriers of *GJB2* mutations.

The fact that none of the patients included in the study had the *GJB6* gene deletions, that we analyzed, implies that further studies on greater populational groups are necessary to evaluate the epidemiologic significance of these mutations in our country.

The deletion del(GJB6-D13S1830), del(GJB6-D13S1854) and del(chr13:19,837,343–19,968,698), specific to West Europe, is otherwise rare or even absent in Eastern Europe countries (rare in Czech population [38,43], absent in Austria [35,44] and Croatia [40,45], etc.).

The findings that the number of deaf persons carrying a single *GJB2* mutation is higher than expected led to a search for other mutations in, or near *GJB2*. As a result there have been identified two large deletions: del(*GJB6*-D13S1830), del(*GJB6*-D13S1854) and the deletion del(chr13:19,837,343–19,968,698) [35]. These deletions truncate the neighboring *GJB6* gene and inhibit the *GJB2* gene expression (by deleting probably a *GJB2* regulatory element not yet identified) so that they may be considered *GJB2* mutations as well. Literature data shows that these mutations are usually found in compound heterozygosity with a *GJB2* coding mutation causing significantly worse HL than most other *GJB2* mutations [31]. One argument for this might be that the expression of both copies of

GJB2 and one copy of *GJB6* is abolished. The del(*GJB6*-D13S1830) deletion seems to be worldwide spread with a much higher occurrence rate than del(*GJB6*-D13S1854) – found mainly in Spain and the UK [31,46]. There are significant more *GJB2* mutation carriers without any of these 2 deletions, indicating that other unidentified mutations/deletions may be present at the *DFNB1* locus, or that another HL cause may be involved.

As to the genotype–phenotype correlation, mention should be made of the fact that even if the literature states that HL caused by connexin 26 mutations is non evolutive [16] – our group includes 2 patients with progressive idiopathic deafness, with early-onset. Progressive hearing deterioration, up to the point where conventional hearing aids become useless (at ages of 6 and respectively 8 years in our group), requires cochlear implantation. The genetic profile of these patients was compound heterozygous in both cases (c.35delG + c.313_326del14 and c.35delG + c.71G>A p.W24*). However, nowadays, more and more authors report cases with SNHL with early-onset and progressive evolution in patients with connexin 26 mutations [17,47].

No possible correlation could be established between deafness degree and genotype, because the studied group consists only of patients with severe to profound SNHL that were cochlear implanted.

At individual level, knowing the genetic etiology is essential for the genetic counseling. There is a 25% recurrence chance for parents that have one child with *GJB2*-related deafness to have another child with the same genotype. Also there is a 66% chance that the second child will have mild-to-moderate HL and a 34% chance that the HL will be more severe if their first child has mild-to-moderate HL. Furthermore, progress made in characterization of genotype–phenotype correlations allows appropriate informing regarding prognostic implications. Several studies have shown that in such a child with severe-to-profound HL that receives a cochlear implant, the parents can expect their child to have an excellent outcome [10]. A genetic diagnosis can be beneficial to parents preventing sometimes parental guilt and anxiety [10,50]. This creates the opportunity to make accurate genetic advice possible and provides prognostic information. Beside the fact that genetic testing has a very important role, test results must be thoroughly interpreted and efforts must be taken to ensure that parents and family members understand the information presented to them. A great deal of attention is needed to take responsibility for these matters.

At a more general level, the knowledge of mutations prevalence in a certain population allows the development of adjusted screening programs.

5. Conclusions

The genetic analysis of *GJB2* mutations revealed that 48.8% of cochlear implanted patients present mutations of connexin 26.

We found an important number of different mutations (6 different mutations) with implications in hearing screening programs development in eastern Romania. The most prevalent mutation of *GJB2* gene was c.35delG mutation. We did not find any new mutation. The connexin 30 studied deletions were not detected in our group.

Conflicts of interest statement

No author has any conflict of interest pertaining to this manuscript.

Acknowledgment

We thank all the subjects who participated in the present study.

References

[1] K.R. White, Early hearing detection and intervention programs: opportunities for genetic services, Am. J. Med. Genet. 130 (2004) 29–36.
[2] R.J.H. Smith, J.F. Dahle, K.R. White, Sensorineural hearing loss in children, Lancet 365 (9462) (2005) 879–890.
[3] P.F. Adams, V. Benson, Current estimates from the National Health Interview Survey, 1990. National Center for Health Statistics, Vital Health Stat. 10 181 (1991) 82–128.
[4] P.E. Mohr, J.J. Feldman, J.L. Dunbar, The societal costs of severe to profound hearing loss in the United States, Int. J. Technol. Assess. Health Care 16 (2000) 1120–1135.
[5] K.R. White, A.B. Maxon, Universal screening for infant hearing impairment: simple, beneficial, and presently justified, Int. J. Pediatr. Otorhinolaryngol. 32 (3) (1995) 201–211.
[6] Joint Committee on Infant Hearing, Year 2007 position statement: principles and guidelines for early hearing detection and intervention programs, Pediatrics 120 (4) (2007) 898–921.
[7] H.A. McPhillips, Early identification and treatment of hearing impairments in children may improve language development, J. Pediatr. 157 (1) (2010) 170–171.
[8] D.M. Houston, R.T. Miyamoto, Effects of early auditory experience on word learning and speech perception in deaf children with cochlear implants: implications for sensitive periods of language development, Otol. Neurotol. 31 (8) (2010) 1248–1253.
[9] M. Manrique, F.J. Cervera-Paz, A. Huarte, M. Molina, Advantages of cochlear implantation in prelingual deaf children before 2 years of age when compared with later implantation, Laryngoscope 114 (8) (2004) 1462–1469.
[10] S.S. Connell, S.I. Angeli, H. Suarez, A.V. Hodges, T.J. Balkany, X.Z. Liu, Performance after cochlear implantation in *DFNB1* patients, Otolaryngol. Head Neck Surg. 137 (4) (2007) 596–602.
[11] A. Parving, Hearing disability in childhood – a cross-sectional and longitudinal investigation of causative factors, Int. J. Pediatr. Otorhinolaryngol. 27 (2) (1993) 101–111.
[12] S. Morzaria, B.D. Westerberg, F.K. Kozak, Systematic review of the etiology of bilateral sensorineural hearing loss in children, Int. J. Pediatr. Otorhinolaryngol. 68 (9) (2004) 1193–1198.
[13] N. Fischel-Ghodsian, T.R. Prezant, W.E. Chaltraw, K.A. Wendt, R.A. Nelson, K.S. Arnos, et al., Mitochondrial gene mutation is a significant predisposing factor in aminoglycoside ototoxicity, Am. J. Otolaryngol. 18 (3) (1997) 173–178.
[14] A. Griffith, T. Friedman, Autosomal and X-linked auditory disorders, in: B. Keats, R. Fay, A. Popper (Eds.), Genetics of Auditory Disorders. Springer Handbook of Auditory Research, vol. 14, 2006, pp. 121–227.
[15] M.B. Petersen, P.J. Willems, Non-syndromic, autosomal recessive deafness, Clin. Genet. 69 (2006) 371–392.
[16] R.L. Snoeckx, P.L. Huygen, D. Feldmann, S. Marlin, F. Denoyelle, J. Waligora, et al., *GJB2* mutations and degree of hearing loss: a multicenter study, Am. J. Hum. Genet. 77 (2005) 945–957.
[17] J.B. Hochman, T.L. Stockley, D. Shipp, V.Y. Lin, J.M. Chen, J.M. Nedzelski, Prevalence of connexin 26 (*GJB2*) and Pendred (SLC26A4) mutations in a population of adult cochlear implant candidates, Otol. Neurotol. 31 (6) (2010) 919–922.
[18] X. Estivill, P. Fortina, S. Surrey, R. Rabionet, S. Melchionda, L. D'Agruma, et al., Connexin-26 mutations in sporadic and inherited sensorineural deafness, Lancet 351 (1998) 394–398.
[19] P.M. Kelley, S. Abe, J.W. Askew, S.D. Smith, S. Usami, W.J. Kimberling, Human connexin 30 (*GJB6*), a candidate gene for nonsyndromic hearing loss: molecular cloning, tissue-specific expression, and assignment to chromosome 13q12, Genomics 62 (1999) 172–176.
[20] N. Hilgert, R.J. Smith, G. Van Camp, Forty-six genes causing nonsyndromic hearing impairment: which ones should be analyzed in DNA diagnostics? Mutat. Res. 681 (2–3) (2009) 189–196.
[21] R. Bruzzone, T.W. White, D.L. Paul, Connections with connexins: the molecular basis of direct intercellular signaling, Eur. J. Biochem. 238 (1996) 1–27.
[22] W.E. Nance, M.J. Kearsey, Relevance of connexin deafness (*DFNB1*) to human evolution, Am. J. Hum. Genet. 74 (6) (2004) 1081–1087.
[23] L. Van Laer, P. Coucke, R.F. Mueller, G. Caethoven, K. Flothmann, S.D. Prasad, et al., A common founder for the 35delG *GJB2* gene mutation in connexin 26 hearing impairment, J. Med. Genet. 38 (8) (2001) 515–518.
[24] P. Gasparini, R. Rabionet, G. Barbujani, S. Melçhionda, M. Petersen, K. Brøndum-Nielsen, et al., High carrier frequency of the 35delG deafness mutation in European populations. Genetic Analysis Consortium of GJB2 35delG, Eur. J. Hum. Genet. 8 (2000) 19–23.
[25] A. Ohtsuka, I. Yuge, S. Kimura, A. Namba, S. Abe, L. Van Laer, et al., *GJB2* deafness gene shows a specific spectrum of mutations in Japan, including a frequent founder mutation, Hum. Genet. 112 (4) (2003) 329–333.
[26] S. Abe, S. Usami, H. Shinkawa, P.M. Kelley, W.J. Kimberling, Prevalent connexin 26 gene (*GJB2*) mutations in Japanese, J. Med. Genet. 37 (1) (2000) 41–43.
[27] Y. Liu, X. Ke, Y. Qi, W. Li, P. Zhu, Connexin26 gene (*GJB2*): prevalence of mutations in the Chinese population, J. Hum. Genet. 47 (2002) 688–690.
[28] R.J. Morell, H.J. Kim, L.J. Hood, L. Goforth, K. Friderici, R. Fisher, et al., Mutations in the connexin 26 gene (*GJB2*) among Ashkenazi Jews with nonsyndromic recessive deafness, N. Engl. J. Med. 339 (1998) 1500–1505.
[29] T. Kudo, K. Ikeda, S. Kure, Y. Matsubara, T. Oshima, K. Watanabe, et al., Novel mutations in the connexin 26 gene (*GJB2*) responsible for childhood deafness in the Japanese population, Am. J. Med. Genet. 90 (2) (2000) 141–145.

[30] M. RamShankar, S. Girirajan, O. Dagan, H.M. Ravi Shankar, R. Jalvi, R. Rangasayee, et al., Contribution of connexin 26 (*GJB2*) mutations and founder effect to nonsyndromic hearing loss in India, J. Mol. Genet. 40 (2003) 68–72.
[31] F.J. del Castillo, M. Rodríguez-Ballesteros, A. Alvarez, T. Hutchin, E. Leonardi, C.A. de Oliveira, et al., A novel deletion involving the connexin-30 gene, del(*GJB6*-d13s1854), found in trans with mutations in the *GJB2* gene (connexin-26) in subjects with *DFNB1* nonsyndromic hearing impairment, J. Med. Genet. 42 (2005) 588–594.
[32] I. del Castillo, M. Villamar, M.A. Moreno-Pelayo, F.J. del Castillo, A. Alvarez, D. Tellería, et al., A deletion involving the connexin 30 gene in non-syndromic hearing impairment, N. Engl. J. Med. 346 (2002) 243–249.
[33] I. del Castillo, M.A. Moreno-Pelayo, F.J. del Castillo, Z. Brownstein, S. Marlin, Q. Adina, et al., Prevalence and evolutionary origins of the del(*GJB6*-D13S1380) mutation in the *DFNB1* locus in hearing impaired subjects: a multicenter study, Am. J. Hum. Genet. 73 (2003) 1452–1458.
[34] R. Birkenhäger, A.J. Zimmer, W. Maier, J. Schipper, Pseudodominants of two recessive connexin mutations in non-syndromic sensorineural hearing loss? Laryngorhinootologie 85 (3) (2006) 191–196.
[35] E. Wilch, H. Azaiez, R.A. Fisher, J. Elfenbein, A. Murgia, R. Birkenhäger, et al., A novel *DFNB1* deletion allele supports the existence of a distant cis-regulatory region that controls *GJB2* and *GJB6* expression, Clin. Genet. 78 (3) (2010) 267–274.
[36] I. Lerer, M. Sagi, Z. Ben-Neriah, T. Wang, H. Levi, D. Abeliovich, A deletion mutation in *GJB6* cooperating with a *GJB2* mutation in trans in non-syndromic deafness: a novel founder mutation in Ashkenazi Jews, Hum. Mutat. 18 (5) (2001) 460.
[37] C. Lazăr, R. Popp, A. Trifa, C. Mocanu, G. Mihut, C. Al-Khzouz, et al., Prevalence of the c.35delG and p.W24* mutations in the *GJB2* gene in patients with nonsyndromic hearing loss from North-West Romania, Int. J. Pediatr. Otorhinolaryngol. 74 (4) (2010) 351–355.
[38] T. Fitzgerald, S. Duva, H. Ostrer, K. Pass, C. Oddoux, R. Ruben, et al., The frequency of *GJB2* and *GJB6* mutations in the New York State newborn population: feasibility of genetic screening of hearing defects, Clin. Genet. 65 (2004) 338–342.
[39] K. Frei, K. Szuhai, T. Lucas, K. Weipoltshammer, C. Schöfer, R. Ramsebner, et al., Connexin 26 mutations in cases of sensorineural deafness in eastern Austria, Eur. J. Hum. Genet. 10 (2002) 427–432.
[40] T. Tóth, S. Kupka, B. Haack, K. Riemann, S. Braun, F. Fazakas, et al., *GJB2* mutations in patients with non-syndromic hearing loss from Northeastern Hungary, Hum. Mutat. 23 (6) (2004) 631–632.
[41] O. Uyguner, M. Emiroglu, A. Uzumcu, Frequencies of gap and tight-junction mutations in Turkish families with autosomal-recessive non-syndromic hearing loss, Clin. Genet. 64 (1) (2003) 65–69.
[42] G. Minarik, V. Ferak, E. Ferakova, A. Ficek, H. Polakova, L. Kadasi, High frequency of *GJB2* mutation W24X among Slovak Romany (Gypsy) patients with non-syndromic hearing loss (NSHL), Gen. Physiol. Biophys. 22 (2003) 549–556.
[43] P. Seeman, O. Bendová, D. Rašková, M. Malíková, D. Groh, Z. Kabelka, 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. 69 (2005) 9–14.
[44] K. Frei, R. Ramsebner, T. Lucas, W.D. Baumgartner, C. Schoefer, F.J. Wachtler, 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. 196 (1–2) (2004) 115–118.
[45] I. Medica, G. Rudolf, M. Balaban, B. Peterlin, C.35delG/*GJB2* and del(*GJB6*-D13S1830) mutations in Croatians with prelingual non-syndromic hearing impairment, BMC Ear Nose Throat Disord. 8 (5) (2005) 11.
[46] N. Pallares-Ruiz, P. Blanchet, M. Mondain, M. Claustres, A.F. Roux, A large deletion including most of *GJB6* in recessive nonsyndromic deafness: a digenic effect? Eur. J. Hum. Genet. 10 (2002) 72–76.
[47] W. Pagarkar, M. Bitner-Glindzicz, J. Knight, T. Sirimanna, Late postnatal onset of hearing loss due to *GJB2* mutations, Int. J. Pediatr. Otorhinolaryngol. 70 (6) (2006) 1119–1124.
[48] F. Denoyelle, D. Weil, M.A. Maw, S.A. Wilcox, N.J. Lench, D.R. Allen-Powell, et al., Prelingual deafness: high prevalence of a 30delG mutation in the connexin 26 gene, Hum. Mol. Genet. 6 (12) (1997) 2173–2177.
[49] D.P. Kelsell, J. Dunlop, H.P. Stevens, N.J. Lench, J.N. Liang, G. Parry, et al., Connexin 26 mutations in hereditary non-syndromic sensorineural deafness, Nature 387 (6628) (1997) 80–83.
[50] F. Denoyelle, S. Marlin, D. Weil, L. Moatti, P. Chauvin, E.N. Garabédian, et al., Clinical features of the prevalent form of childhood deafness, DFNB1, due to a connexin-26 gene defect: implications for genetic counselling, Lancet 353 (9161) (1999) 1298–1303.