Prevalence of the GJB2 mutations and the del(GJB6-D13S1830) mutation in Brazilian patients with deafness

Vânia Belintani Piatto a,*, Eny Maria Goloni Bertollo a,1, Edi Lúcia Sartorato b,2, José Victor Maniglia a,3

a Medical School of São José do Rio Preto (FAMERP), Rua Frei Baltazar, No. 415, Boa Vista, São José do Rio Preto, São Paulo 15025-390, Brazil
b Molecular Biology Center and Genetics Engineering (CBMEG) of State University of Campinas (UNICAMP), Cidade Universitária Zeferino Vaz s/n., Barão Geraldo, Campinos, São Paulo 13083-970, Brazil

Received 27 December 2003; accepted 25 May 2004

Abstract

Mutations in the GJB2 gene are the most common cause of sensorineural non-syndromic deafness in different populations. One specific mutation, 35delG, has accounted for the majority of the mutations detected in the GJB2 gene in many countries. The aim of this study was to determine the prevalence of GJB2 mutations and the del(GJB6-D13S1830) mutation in non-syndromic deaf Brazilians. The 33 unrelated probands were examined by clinical evaluation to exclude syndromic forms of deafness. Mutation analysis in the GJB2 gene and the testing for the del(GJB6-D13S1830) were performed in both the patients and their family members. The 35delG mutation was found in nine of the probands or in 14 of the mutated alleles. The V37I mutation and the del(GJB6-D13S1830) mutation were also found in two patients, both are compound heterozygote with 35delG mutation. These findings strengthen the importance of genetic diagnosis, providing early treatment, and genetic counseling of deaf patients. © 2004 Published by Elsevier B.V.

Keywords: Hearing loss; Molecular analysis; Connexin 26; 35delG mutation, del(GJB6-D13S1830) mutation

1. Introduction

Surveys show that more than 70 million people worldwide have hearing loss that affects normal communication. In developed countries, the incidence of congenital severe hearing impairment is 1 in 1000 births, half of which can be attributed to genetic factors (Marazita et al., 1993). Genetic heterogeneity and environmental factors have impaired identification of the genes causing deafness until recently (Mustapha et al., 2001). In Brazil, most cases of hearing loss are due to environmental factors, such as congenital infections (mainly rubella), perinatal anoxia and meningitis (Simões and Maciel-Guerra, 1992).

About 70% of cases of hereditary pre-lingual deafness belong to the non-syndromic form and are believed to result from a sensorineural (cochlear) defect. Within the non-syndromic hearing loss category, 75–80% of cases of congenital pre-lingual deafness are inherited in an
autosomal recessive way, followed by dominant (15–20%) and X-linked (1–1.5%) manners (Nance, 2003). Deafness is an extremely genetically heterogeneous disorder, shown by the fact that 33 loci for recessive non-syndromic hearing loss (recessive locus has the prefix “DFNB”) and 39 loci for dominant non-syndromic hearing loss have been mapped to date (update regularly on the Hereditary Hearing Loss Homepage (HHH); http://dnalab-www.uia.ac.be/dnalab/hhh/index.html.

The most common mutation associated with DFNB1 hearing loss, responsible for most (up to 85%) of the mutants alleles in Europe–Mediterranean populations is a deletion of a guanine from a series of six guanines extending from nucleotide position 30–35 (35delG) in the coding region of the connexin 26 gene, GJB2. This leads to a frameshift and a resulting stop codon at position 13 (Zelante et al., 1997). Many studies from various parts of the world have documented the incidence of GJB2 mutations in the deaf population. These include France, Italy, Spain, UK, the United States, Israel, and most recently, Lebanon, Greece, Austria, China, Brazil, and the Iranian and Palestinian populations (Kelley et al., 1998; Green et al., 1999; Sobe et al., 2000; Mustapha et al., 2001; Frei et al., 2002; Liu et al., 2002; Najmabadi et al., 2002; Oliveira et al., 2002; Pampanos et al., 2002; Shahin et al., 2002).

In several European countries, the prevalence of the 35delG mutation has been estimated to 2–4% of the population with normal hearing (Estivill et al., 1998; Gasparini et al., 2000). In Brazil, the 35delG carrier frequency in the white population with normal hearing in the Southeast region (1 in 51) (Oliveira et al., 2004) was similar to that in the overall European population (Gasparini et al., 1997). In fact, in a previous study performed in Brazil, six 35delG heterozygotes were identified among 620 randomly selected neonates; a 35delG carrier rate of 0.97% (1 in 103), showing that this mutation in not rare in the Brazilian population (Sartorato et al., 2000). In spite of the fact that all patients studied were Caucasian, the composition of the Brazilian population is difficult to be established, due to its high ethnic composition of Caucasian, African and Amerindian origin.

It is worth noticing that for several deaf patients, a Cx26 mutation was detected on one allele only, indicating either the existence of another Cx26 mutation in the gene unexplored region or the possible complication of another connexin gene for a digenic origin of the hearing loss, which could be related to the putative formation of heteromeric connexons or heterotypic channels, such as Cx26 and Cx32 or as Cx26 and Cx30 (Ahmad et al., 1999; Kumar, 1999; Lautermann et al., 1999; Marziano et al., 2003). These cases accounted for 10–42% of all deaf subjects with a least one GJB2 mutation (Wilcox et al., 2000). Perhaps, these findings could be also attributed to others mutations that might exist in the DFNB1 locus, but not in the GJB2 gene, which could provide an explanation for the high proportion of the heterozygotes deaf subjects. Recently, this hypothesis received experimental support by the finding of a novel class of mutations in the DFNB1 locus, which does not affect GJB2, but truncates the neighboring GJB6 gene, which encodes Cx30 (Grifa et al., 1999; Lerer et al., 2001; del Castillo et al., 2002; Pallares-Ruiz et al., 2002). In one study, the deletion breakpoint junction was isolated and sequenced, revealing the loss of DNA segment of approximately 342 Kb, with one breakpoint inside the GJB6 coding region. This deletion, named del(GJB6-D13S1830) was the accompanying mutation in about 50% of these deaf GJB2 patients (del Castillo et al., 2002).

In this study, we assessed the prevalence of GJB2 mutations in Brazilian patients with non-syndromic sensorineural hearing loss and we determined the types of mutations in this population. Based on the association found between GJB2 monoallelic mutations and the del(GJB6-D13S1830) mutation (Grifa et al., 1999; Lerer et al., 2001; del Castillo et al., 2002; Pallares-Ruiz et al., 2002), we also investigated the contribution of this deletion to hearing impairment in this population.

2. Subjects and methods

From March 2002 to June 2002, the study was conducted on 33 unrelated probands with congenital non-syndromic sensorineural hearing loss, referred to from the Otorhinolaryngology Service of Medical School of São José do Rio Preto (FAMERP), São Paulo, Brazil. Deaf subjects aged 1–37 years (mean 24.2), 23 males and 10 females, monitored at least twice a year since the diagnosis in the same clinical and audiological institution (FAMERP), were included in the study. Written informed consent was obtained from the patients or from the parents in case of underaged ones. A detailed history was obtained for each subject. A clinical evaluation was performed by the same otorhinolaryngologist and pediatrician, and an audiometric assessment was performed by the same audiologist, together with a molecular analysis of the complete sequence of GJB2 gene.

2.1. Clinical evaluation

In each patient, a complete medical history was obtained to record age of onset of deafness, and to exclude the possibility of environmental and syndromic causes: (1) history or signs of infections during pregnancy (STORCH + HIV); (2) birth weight < 1,500 g; (3) neonatal Apgar scores < 0–4 at one minute or < 0–6 at five minute; (4) need of neonatal mechanical ventilation; (5) hyperbilirubinaemia requiring transfusion; (6) use of
ototoxic medication (aminoglycosides, antibiotics, etc.) in multiple courses; (7) bacterial meningitis or other infections associated with sensorineural impairment; (8) history of head trauma associated with skull fractures or loss of consciousness; (9) signs of neurodegenerative disorders.

The patients also underwent an otoscopic and general examination with a systematic search to exclude suggestive signs of a syndromic form of deafness (especially dysmorphism, integumentary disorders, and branchial, cardiac, and thyroid anomalies). They also had an ophthalmological evaluation (including fundoscopy), and search for haematuria and proteinuria, and vestibular tests (eye-tracking tests for spontaneous nystagmus, or gaze-evoked nystagmus, and caloric tests). High-resolution temporal bone computerized tomography with axial and coronal sections of 1 mm was carried out in each patient in order to exclude those with temporal bone and inner ear malformation.

Therefore, all this clinic evaluation was carried out to exclude the patients with hearing impairment caused by environmental factors, congenital malformation of inner ear, or by genetic syndromes.

Thus, all patients included in the study have a bilateral sensorineural non-syndromic hearing loss classified as mild (25–40 dB), moderate (41–60 dB), severe (61–80 dB) or profound (>81 dB) (World Health Organization, 1991), and of unknown etiology and without congenital malformation.

After written informed consent, both family members with normal audition or with deafness (familial recurrence) of unrelated probands who tested positive for Cx26 mutations underwent a molecular evaluation.

2.2. Molecular analysis

Molecular analysis was performed at the Molecular Biology Center and Genetics Engineering – CBMEG of State University of Campinas (UNICAMP), São Paulo, Brazil.

DNA was extracted from EDTA-anticoagulated whole blood samples using the GFX™ Genomic Blood DNA Purification Kit (Amersham Pharmacia Biotech Inc. Limited 2000) according to the manufacturer’s protocol.

All DNA samples were screened for the 35delG mutation using primers and conditions for allele-specific PCR (AS-PCR) as previously described (Scott et al., 1998), including a primer set of the X–Y homologous gene amelogenin as the internal reaction control (Antoniadi et al., 1999). The products of AS-PCR were electrophoresed on 1.5% agarose gel and visualized with ethidium bromide staining under ultraviolet light to verify their size and quantity.

The AS-PCR method was designed to identify the 35delG mutation, and to discriminate between the normal and mutant alleles. By running two reactions, with normal or mutant primers on each sample it was possible to distinguish between homozygotes non-35delG individuals, homozygotes 35delG individuals and 35delG carriers.

In order to screen the del(GJB6-D13S1830) mutation, we used primers and conditions for PCR as previously reported (del Castillo et al., 2002), as well as a modification of the method to positively detect a wild-type product by adding another primer that is located in the deleted segment of GJB6 (Grifa et al., 1999). Using the primers together, two different PCR products were obtained, providing discrimination of wild-type subjects (500 bp products), homozygotes for the deletion (396 bp products), and heterozygotes (both products) in a single test. The products of PCR were electrophoresed on 1.5% agarose gel and visualized with ethidium bromide staining under ultraviolet light to verify their size and quantity.

Patients heterozygotes for the 35delG mutation, patients with no detectable 35delG mutation, and those patients who had no detectable del(GJB6-D13S1830) mutation were further analyzed by direct sequencing (Sanger and Coulson, 1975) of the coding region of the connexin 26 (GJB2) gene. These DNA samples underwent a PCR method for direct sequencing, using forward and reverse primers in conditions previously described (Kelsell et al., 1997; Kelley et al., 1998). The products were used to produce a template for sequencing, and they were purified using the Kit Wizard SV Gel and PCR Clean-up System™ (PROMEGA) according to the manufacturer’s protocol. They were sequenced with the Applied Biosystems (ABI) Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit™ and ABI Prism model 377 DNA sequencer™ (Perkin–Elmer Applied Biosystem). All PCR products were sequenced in both directions. When after that, no mutations were found, the splice site was amplified and PCR products were sequenced. Experimental conditions for PCR and sequencing were as previously described (Denoyelle et al., 1999; Green et al., 1999).

2.3. Ethical approval

The study was approved by the Ethics in Human Research Committee of the Medical School of São José do Rio Preto (FAMERP), São Paulo, Brazil, and by Ethics in Human Research National Committee of Brasilia (Federal District), Brazil.

3. Results

Mutations in the GJB2 gene were found in nine of the unrelated probands in this studied population. Five subjects of this hearing impairment population were
homozygotes for the most frequent mutation, the deletion named 35delG. This mutation accounted in 14 out of 66 of the GJB2 analyzed alleles, and in 14 out of 16 of the GJB2 mutated alleles. Four heterozygotes for the 35delG mutation were identified, but the second GJB2 mutation was detected in only one of them (35delG/V37I) by direct sequencing of the entire coding region of the GJB2 gene, and the del(GJB6-D13S1830) mutation was also detected in only one of them [35delG/del(GJB6-D13S1830)], by PCR testing. In 24 unrelated probands no changes in the alleles were found, by direct sequencing of the entire coding region of the GJB2 gene, neither in the splice site. Molecular data from all unrelated probands, their parents and relatives are presented in Table 1.

To study a possible correlation between genotype and phenotype in patients with GJB2 mutations, we grouped the 33 unrelated probands according to the severity of

Table 1
Genotypes of the unrelated probands, parents and relatives

<table>
<thead>
<tr>
<th>Probands</th>
<th>Mutations</th>
<th>Parents</th>
<th>Deaf relatives</th>
<th>Mutations</th>
<th>Normal hearing relatives</th>
<th>Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Allele 1/Allele 2</td>
<td>Father</td>
<td>Allele 1/Allele 2</td>
<td>Mother</td>
<td>Allele 1/Allele 2</td>
<td>Allele 1/Allele 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>35delG/V37I</td>
<td>35delG/N</td>
<td>V37I/N</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>N/N</td>
<td>NA</td>
<td>NA</td>
<td>Brother</td>
<td>N/N</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>N/N</td>
<td>X</td>
<td>NA</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>N/N</td>
<td>X</td>
<td>x</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>N/N</td>
<td>NA</td>
<td>NA</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>N/N</td>
<td>X</td>
<td>NA</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>N/N</td>
<td>NA</td>
<td>NA</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
<td>N/N</td>
<td>X</td>
<td>NA</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>9</td>
<td>N/N</td>
<td>X</td>
<td>NA</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>10</td>
<td>35delG/35delG</td>
<td>X</td>
<td>35delG/N</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>11</td>
<td>N/N</td>
<td>NA</td>
<td>NA</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>12</td>
<td>N/N</td>
<td>NA</td>
<td>NA</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>13</td>
<td>N/N</td>
<td>NA</td>
<td>NA</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>14</td>
<td>N/N</td>
<td>X</td>
<td>x</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>15</td>
<td>35delG/35delG</td>
<td>X</td>
<td>35delG/N</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>16</td>
<td>N/N</td>
<td>X</td>
<td>x</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>17</td>
<td>N/N</td>
<td>X</td>
<td>x</td>
<td>Sister</td>
<td>N/N</td>
<td>–</td>
</tr>
<tr>
<td>18</td>
<td>N/N</td>
<td>X</td>
<td>x</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>19</td>
<td>N/N</td>
<td>NA</td>
<td>NA</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>20</td>
<td>35delG/N</td>
<td>X</td>
<td>x</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>21</td>
<td>35delG/35delG</td>
<td>X</td>
<td>35delG/N</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>22</td>
<td>35delG/35delG</td>
<td>35delG/N</td>
<td>35delG/N</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>23</td>
<td>35delG/35delG</td>
<td>35delG/N</td>
<td>NA</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>24</td>
<td>N/N</td>
<td>X</td>
<td>x</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>25</td>
<td>N/N</td>
<td>NA</td>
<td>N/N</td>
<td>Mother</td>
<td>N/N</td>
<td>–</td>
</tr>
<tr>
<td>26</td>
<td>N/N</td>
<td>X</td>
<td>x</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>27</td>
<td>N/N</td>
<td>NA</td>
<td>NA</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>28</td>
<td>35delG/Δ(GJB6-D13S1830)</td>
<td>35delG/N</td>
<td>35delG/N</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>29</td>
<td>N/N</td>
<td>X</td>
<td>x</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>30</td>
<td>N/N</td>
<td>NA</td>
<td>N/N</td>
<td>Mother</td>
<td>N/N</td>
<td>–</td>
</tr>
<tr>
<td>31</td>
<td>35delG/N</td>
<td>NA</td>
<td>NA</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>32</td>
<td>N/N</td>
<td>NA</td>
<td>NA</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>33</td>
<td>N/N</td>
<td>NA</td>
<td>NA</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

N – No mutations in the GJB2 gene and/or without the del(GJB6-D13S1830) mutation; NA – not analysed; x – samples unauthorized.
the hearing loss. Among the five unrelated probands with 35delG homozygosity, one had profound hearing loss, three had severe, and one moderate hearing loss. Among the four patients with 35delG heterozygosity, one compound heterozygote (35delG/V37I), and one compound heterozygote [35delG/del(GJB6-D13S1830)], and one carrier proband (35delG/No mutation) had severe hearing loss, and the other carrier proband had moderate hearing loss.

4. Discussion

Even with this study that has been carried out with a small number of samples, mutations in the GJB2 gene were found in nine of unrelated probands in this study and, the 35delG mutation accounted in fourteen of the mutated alleles. Our results are in accordance with a previous study in which the 35delG mutation was found in 16 of the mutated alleles in Brazilian patients with non-syndromic hearing loss resulting from mutations in the GJB2 gene (Oliveira et al., 2002) and are also in accordance with many others studies which documented the incidence of the GJB2 mutations in patients with hearing impairment (Kelley et al., 1998; Green et al., 1999; Sobe et al., 2000; Mustapha et al., 2001; Frei et al., 2002; Liu et al., 2002; Najmabadi et al., 2002; Pampananos et al., 2002; Shahin et al., 2002).

Another change observed in the GJB2 gene, V37I mutation (case 1 – 35delG/V37I) was first described as a polymorphism in a US population where it did not segregate with deafness (Kelley et al., 1998), with no functional activity. These data rather suggest that V37I causes hearing loss and corroborate a more recent mutational analysis (Wilcox et al., 2000) that identified two individuals with sensorineural deafness: one was homozygote for this mutation, whereas the other was a compound heterozygote with the commonest pathological mutation, 35delG, according to the present study. The 109G→A mutation involves the conversion of a valine at codon 37 to isoleucine in the first transmembrane domain of connexin 26. A valine at codon 37 is invariant in β connexins and absent in α connexins, in which glycine, serine and alanine are found at that position (Kelley et al., 1998). The compound heterozygosity with 35delG inherited from the father, and V37I inherited from the mother led to non-syndromic recessive deafness in the patient of this study.

Testing for the del(GJB6-D13S1830) mutation was performed, and the deletion accounted for 25% of heterozygotes for the 35delG mutation, according to the previous study (del Castillo et al., 2002; del Castillo et al., 2003; Pandya et al., 2003; Wu et al., 2003). This finding may indicate a digenic pattern of inheritance of hearing impairment from mutations involving GJB2 and GJB6. Thus, the pathophysiological hypotheses concerning the Cx26 and Cx30 associated auditory defects are similar, and the disruption of these heterotypic channels or heteromeric connexons by certain mutations may underlie the non-syndromic nature of the deafness (Petit et al., 2001). Therefore, heterozygotes at the GJB2 locus should be screened for the del(GJB6-D13S1830) mutation as a cause of deafness (Stevenson et al., 2003), according to the present study.

The connexins 26 mutations have been mainly reported in patients with deafness, ranging from mild to profound (Denoyelle et al., 1999; Cohn et al., 1999; Cohn and Kelley, 1999b; Mueller et al., 1999; Walch et al., 2000; Wilcox et al., 2000; Engel-Yeger et al., 2002; Cryns et al., 2004). In this study was found a range of hearing-deaf individuals between mild to profound deafness, according to the previous studies.

In our study, we could not detect a mutation in other allele in two individuals and in both alleles in 24 individuals. Some studies support the hypothesis that other factors may modify the phenotypic effects of mutations in the GJB2 gene, as the existence of modifier genes has been known for a long time. One other possibility is that in these individuals the hearing loss is due to a mutation in an entirely different gene (Estivill et al., 1998).

The search for mutations in GJB2 gene is important for public health issue and genetic counseling. The proportion of genetic causes tends to increase as a result of improvements in health care in developing countries, and the molecular diagnostic test may provide the determination of a genetic origin in these countries. The 35delG mutation is easy to detect and the test would be practicable to distinguish the common heterozygotes carriers for Cx26 mutations from either DFNB1-deaf heterozygotes individuals or DFNB1-deaf homozygotes individuals (Denoyelle et al., 1999; Van Camp, 2002).

There is considerable interest in establishing the prevalence and types of mutations causing non-syndromic hearing loss in Brazil. This information will allow the design of simple, more specific and cheaper assays for the detection of 35delG mutation, according to the present study. Identification of the underlying genetic causes of hearing loss is central to improved counseling of families with deaf members.

Acknowledgements

We would like to thank the families who participated in this work. We further thank Camila Andräa Oliveira and Fabiana Alexandino from UNICAMP, and Carla Renata Graça, Ellen Caroline Toledo do Nascimento and Érika Cristina Pavarino Bertelli, from FAMERP for their help and support in this work.
References

253.


Cohn, E.S., Kelley, P.M., 1999b. Clinical phenotype and mutations in
connexin 26 gene (GJB2) that cause autosomal recessive (DFNB1)

Kelley, P.M., Harris, D.J., Comer, B.C., Askew, J.W., Fowler, T.,
Smith, S.D., Kimberling, W., 1998. Novel mutations in the connexin 26 gene (GJB2) that cause autosomal recessive (DFNB1)

Kelsell, D.P., Dunlop, J., Stevens, H.P., Lench, N.J., Liang, J.N.,
in hereditary non-syndromic sensorineural deafness. Nature 387,
80–83.

Kumar, N.M., 1999. Molecular biology of the interactions between

Lautermann, J., Frank, H.G., Jahne, K., Traub, O., Winterhager, E.,
1999. Developmental expression patterns of connexin 26 and 30 in

Lerer, I., Sagi, M., Ben-Neriah, Z., Wang, T., Levi, H., Abeloivici,
D., 2001. A deletion mutation in GJB6 cooperating with a GJB2
mutation in non-syndromic deafness: a novel founder mutation in
Ashkenazi jews. Hum. Mutat. 18, 460.

et al., 2002. The prevalence of connexin 26 (GJB2) mutations in

Marazita, M.L., Ploughman, L.M., Rawlings, B., Remington, E.,
Arnos, K.S., Nance, W.E., 1993. Genetic epidemiological studies of
Genet. 46, 486–491.

Marziano, N.K., Casalotti, S.O., Portelli, A.E., Becker, D.L., Forge,
A., 2003. Mutations in the gene for connexin 26 (GJB2) that cause
hearing loss have a dominant negative effect on connexin 30. Hum.

Mueller, R.F., Nehammer, A., Middleton, A., Houseman, M., Taylor,
sensorineural hearing impairment due to connexin 26 gene muta-
tions – molecular and audiological findings. Int. J. Ped. Otomho-
rolaryngol. 50, 3–13.

Mustapha, M., Salem, N., Delague, V., Chouery, E., Ghassibeh,
recessive non-syndromic hearing loss in the Liba-
nese population: prevalence of the 30delG mutation and report of
two novel mutations in the connexin 26 (GJB2) gene. J.

Najmabadi, H., Cucci, R.A., Sahebjam, S., Kouchakian, N., Farhadi,
M., Kahrizi, K., et al., 2002. GJB2 mutations in Iranians with
autosomal recessive non-syndromic sensorineural hearing loss.
Hum. Mutat. 19, 572–577.


Oliveira, C.A., Alexendrino, F., Abe-Sandes, K., Silva Jr., W.A.,
Maciel-Guerra, A.T., 2002. Deafness resulting from mutations in
the connexin 26 (GJB2) gene in Brazilian patients. Clin.
Genet. 61, 354–358.

Pallares-Ruiz, N., Blanchet, P., Mondain, M., Claustes, M., Roux,
A.F., 2002. A large deletion including most of GJB6 in recessive non-

Pampanos, A., Economides, J., Iliadou, V., Neou, P., Leotsakos,
prelingual deafness in the Greek population. Int. J. Pediatr.
Otorhinolaryngol. 65, 101–108.

Pandya, A., Arnos, K.S., Xia, X.J., Welch, K.O., Blanton, S.H.,
Friedman, T.B., et al., 2003. Frequency and distribution of GJB2
(connexin 26) and GJB6 (connexin 30) mutations in a large North

Petit, C., Levilliers, J., Hardelin, J.-P., 2001. Molecular genetics of

DNAs in DNA by primed synthesis with DNA polymerase. J.
Mol. Biol. 94, 444–448.

Sartorato, E.L., Gottardi, E., Oliveira, C.A., Magna, L.A., Annichino-
resulting from mutations in the GJB2 (connexin 26) gene in
Genet. 37, 444–448.

Sartorato, E.L., Gottardi, E., Oliveira, C.A., Magna, L.A., Annichino-
mination of the frequency of the 35delG allele in Brazilian


