

Detection of 35delG, 167delT mutations in the connexin 26 gene among Egyptian patients with nonsyndromic sensorineural hearing loss

Nehal E. El Barbary^a, Mervat F. El Belbesy^a, Samir I. Asal^b, Soha F. Kholeif^a

^aDepartment of Human Genetics, Medical Research Institute, ^bDepartment of Audiology Unit, Otorhinolaryngology, Faculty of Medicine, Alexandria University, Alexandria, Egypt

Correspondence to Nehal El Sayed El Barbary, MS, Human Genetics Department, Medical Research Institute, Alexandria University, 21561 Alexandria, Egypt
Tel: +20 1224100640; fax: (+203) 4283719; e-mail: nehal.elbarbary@yahoo.com

Received 10 December 2013

Accepted 08 May 2014

The Egyptian Journal of Otolaryngology
2015, 31:42–46

Aim

The aim of this study was to detect 35delG and 167delT mutations in the *connexin 26 gene* among Egyptian patients with nonsyndromic sensorineural hearing loss, allowing accurate diagnosis, proper genetic counseling, and carrier detection.

Patients and methods

Fifty-one patients were subjected to 35delG and 167delT mutations detection using PCR-based techniques.

Results

Seven patients had the 35delG mutation. Four patients were homozygous and four patients were heterozygous for this deletion. Two homozygotes were sibs and two heterozygotes were sibs as well. The allelic frequency for 35delG was 10.8%. The 167delT was not detected in any of the patients studied.

Conclusion

The 35delG is a common pathogenic mutation and an important contributor toward autosomal recessive nonsyndromic hearing loss in the Egyptian population.

Keywords:

167delT mutation, 35delG mutation, SNHL

Egypt J Otolaryngol 31:42–46

© 2015 The Egyptian Oto - Rhino - Laryngological Society
1012-5574

Introduction

Congenital hearing loss affects ~1–3 in 1000 live births in the general population, and 50–60% of these cases have genetic etiologies [1]. Nonsyndromic deafness is responsible for about 70% of the genetic deafness cases involving more than 100 different genes with patterns of autosomal recessive, autosomal dominant, X-linked, and mitochondrial inheritance, the autosomal recessive form being the most common one in ~77% of the patients [2]. Despite this genetic heterogeneity, deafness related to the DFNB1 locus is the most common cause of autosomal recessive nonsyndromic hearing loss (ARNSHL) in many countries worldwide. DFNB1 is the first locus incriminated in ARNSHL and it includes the Gap junction $\beta 2$ (*GJB2*) gene [3].

GJB2 is a small gene situated on chromosome 13q11 with two exons (only exon two contains the coding sequence). It encodes connexin (Cx) 26 protein of 226 amino acids [4]. Cx 26 is the predominant Cx expressed in the cochlea. It has been proposed that Cx 26 plays a major role in the recycling of potassium ions from hair cells to the stria vascularis, where they are actively pumped back into the cochlear endolymph, and this is essential for the auditory function [5]. Mutations in *GJB2* explain more than 50% of nonsyndromic recessive hearing loss cases in most populations worldwide [6].

In the *GJB2* gene, over 100 different mutations (mostly recessive) are found to be associated with deafness and they have ethnic and geographical differences [7].

The 35delG is the most common recessive mutation in the Whites and may cause up to 70% of all *GJB2* gene mutations. This frameshift mutation is caused by the deletion of a guanine base in a sequence of six guanines, which extends from nucleotide position 30–35 in the coding region of the *GJB2* gene, resulting in the synthesis of a truncated nonfunctioning polypeptide with 12 amino acids, as opposed to the wild-type protein that contains 226 amino acids [8].

The 167delT is the second most frequently found mutation in individuals with the autosomal recessive form of nonsyndromic deafness caused by the *GJB2* gene. This mutation is common in affected Ashkenazi Jews, and is found sporadically in some other populations [2].

Early diagnosis of *GJB2* mutations in nonsyndromic deaf patients is crucial for prognosis and appropriate management. It may also help public health services to adopt effective preventive measures for carrier detection and proper genetic counseling [9].

The aim of this study was to detect the frequency of 35delG and 167delT mutations in the *Cx 26* gene

among patients with nonsyndromic sensorineural hearing loss, enabling accurate diagnosis, more effective management, proper genetic counseling, and carrier detection.

Patients and methods

This study was carried out on 51 Egyptian patients (from 40 families) referred to the Human Genetics Department, Medical Research Institute, from the Audiology Unit, Faculty of Medicine, Alexandria University. An informed consent was obtained from the parents of each participant.

The evaluation for the study group included a detailed genetic history, assessment of peripheral hearing sensitivity using pure tone audiometry or auditory brainstem response, and otorhinolaryngologic and clinical examination. Other serologic and radiologic investigations were performed to exclude acquired and syndromic causes of hearing loss.

The study group that was subjected to molecular testing included patients with bilateral, prelingual, nonsyndromic, and sensorineural hearing loss, with no definite known acquired etiology such as infections, teratogens, acoustic trauma, or any other neonatal disease, and no limitations in terms of age or sex.

Molecular genetic study

Genomic DNA was extracted from peripheral blood leukocytes using the QIAamp DNABlood Mini Kit (Qiagen, Germany) according to the manufacturer’s instructions. All DNA samples were screened for the 35delG by PCR-mediated site-directed mutagenesis, followed by *BsI* digestion [10], and for the 167delT by PCR, followed by *PstI* digestion [11] (Table 1).

Results

The age range of the deaf patients was 3 months to 24 years, mean age 5.4 years (SD = 4.1). Twenty-nine (56.9%) of the 51 tested patients were males and 22 (43.1%) were females.

Seven patients had the 35delG mutation (7/51, 13.7%) among five families. Four patients were homozygous

and three patients were heterozygous for the deletion (Table 2). Two homozygotes for this mutation were sibs and two heterozygotes were sibs as well. The allelic frequency for 35delG was 10.8% (11/102 investigated alleles in 51 patients) (Fig. 1).

The seven 35delG homozygote and heterozygote patients had severe to profound sensorineural hearing loss (SNHL). All the seven homozygous and heterozygous 35delG patients were born to consanguineous parents. The 35delG homozygous patients were two male and two female patients. The 35delG heterozygous patients were three females.

For the deaf patients homozygous for the deletion (three families), all their normal hearing parents were identified to be heterozygous carriers for the 35delG. In one family, a normal hearing maternal aunt was a carrier for 35delG. In another family, a normal hearing sib was not a carrier.

For the deaf patients heterozygous for the deletion (two families), the parents in one family refused to undergo molecular testing. In the other family, the normal hearing mother was identified to be a heterozygous carrier for the 35delG, whereas the normal hearing father was not a carrier.

The 167delT mutation was not detected in any of the patients studied (Fig. 2).

Discussion

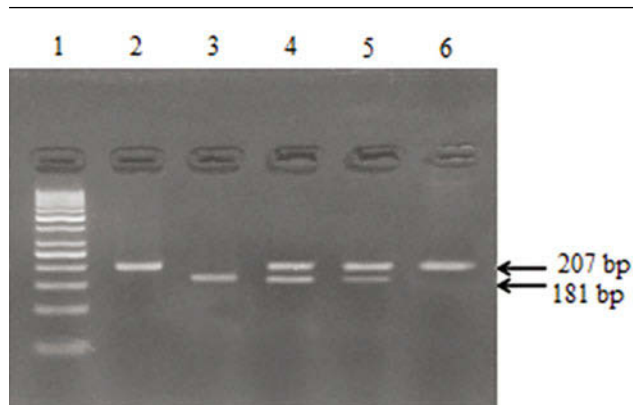
Advancements in genomics have been crucial in clarifying the genetic heterogeneity of nonsyndromic hearing impairment [12]. Determination of the common mutations in a specific country allows for the specific use of genetic testing for carrier screening and diagnostic purposes [13].

The data in the present study suggest that the 35delG is an important mutation related to ARNSHL in the Egyptian population. In the current study, the allele frequency of 35delG was found to be 10.8%, which is in agreement with that reported previously by Snoeckx *et al.* [14], who carried out a study on 159 Egyptian patients from 111 families with nonsyndromic bilateral SNHL, and the allele frequency detected was 10.8%. The allele frequency of 35delG in the present study

Table 1 Polymerase chain reaction /restriction fragment length polymorphism for the detection of 35delG and 167delT mutations [10,11]

| Mutation | PCR product (bp) | Restriction enzyme | Digest homozygous normal (bp) | Digest homozygous Mutant (bp) | Digest heterozygous carrier (bp) |
|----------|------------------|--------------------|-------------------------------|-------------------------------|----------------------------------|
| 35delG | 207 | <i>BsI</i> | 207 | 181,26 | 207,181,26 |
| 167delT | 272 | <i>PstI</i> | 112,91,69 | 181,91 | 181,112,91,69 |

Figure 1



Restriction digestion of PCR products of the GJB2 gene for the detection of the 35delG mutation using *BstI* enzyme resolved on a 3.5% agarose gel. Lane1: molecular weight marker (50 bp DNA ladder); lane 2: 207 bp PCR product; lane 3: digest product for a deaf patient identified to be homozygous for the 35delG mutation showing a band at 181 bp (band at 26 bp could not be resolved on a 3.5% agarose gel); lanes 4 and 5: digestion products for deaf patients identified to be heterozygous carriers for the 35delG mutation showing bands at 181 and 207 bps (bands at 26 bp could not be resolved on a 3.5% agarose gel); lane 6: digestion product for a deaf patient negative for the 35delG mutation showed a band at 207 bp.

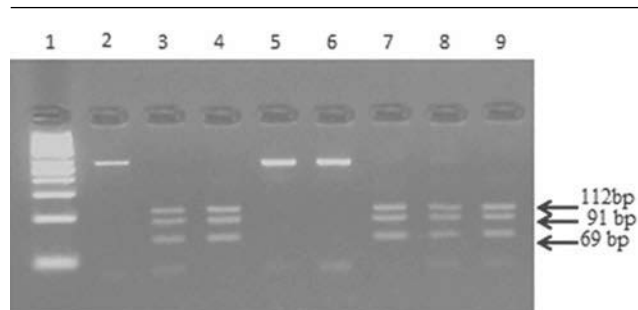
Table 2 Genotype frequencies for the 35delG mutation in the patients studied

| Genotypes | Patients [N (%)] |
|--|------------------|
| Detected 35delG mutation | |
| Homozygote (35delG/35delG) | 4 (7.8) |
| Heterozygote (35delG/non-35delG) | 3 (5.9) |
| No mutation detected (non-35delG/non-35delG) | 44 (86.3) |
| Total | 51 (100) |

was concordant with the allele frequency reported by Abdel-Meguid *et al.* [15] (10.17%). However, Mohamed *et al.* [16] carried out a study on 155 Egyptian individuals from 97 families and the 35delG was detected in 27/310 (8.7%) investigated alleles.

Furthermore, the results of the present study were in agreement with those reported by authors from Turkey, Jordan, and Palestine. In a Turkish study by Evirgen *et al.* [17] carried out on 47 patients, the allele frequency of the 35delG was determined to be 11.7%. Similar results were obtained in a Jordanian study [18] in which the allele frequency detected was 11.5%. In a Palestinian study carried out by Shahin *et al.* [19] on 48 patients, the allele frequency for 35delG was 14%. This frequency is higher than that reported in the current study. This could be attributed to the difference in the selection criteria of both study groups. The deaf patients included in the Palestinian study had bilateral, prelingual, nonsyndromic hearing loss of more than 85 dB (severe hearing loss), whereas in the present study, the tested patients had moderate to profound, bilateral, prelingual, nonsyndromic hearing loss.

Figure 2



PCR/restriction fragment length polymorphism of the GJB2 gene in deaf patients for the detection of the 167delT mutation using *PstI* enzyme resolved on a 3.5% agarose gel. Lane1: molecular weight marker (50 bp DNA ladder); lanes 2, 5, and 6: 272 bp PCR products; lanes 3, 4, 7, 8, and 9: digestion products for deaf patients negative for the 167delT mutation (wild genotypes) showed bands at 69, 91, and 112 bp.

The frequency of 35delG in the Moroccan and Tunisian population is greater than that reported in Egypt. This may be because of the immigration patterns responsible for the 35delG frequencies in the Mediterranean countries. Gazzaz *et al.* [20] reported a 35delG allele frequency of 21.55% in their study on Moroccan patients, whereas Trabelsi *et al.* [21] reported an allele frequency of 23.68% for the 35delG in Tunisian deaf patients.

The present data showed that the 35delG frequency in Egypt is less than that reported from different populations of European descent. Owing to the single origin of the 35delG and the migration patterns, the highest allele frequencies for the 35delG were reported in Southern European countries such as Greece and Italy, and they are higher than those detected in Northern European countries such as Germany.

In a Greek study by Pampanos *et al.* [22], the detected allele frequency for the 35delG was 33.1% among 210 deaf patients. Murgia *et al.* [23] carried out a study on 53 Italian patients in which the allele frequency was 37.7%. In a German study carried out by Gabriel *et al.* [24], the allele frequency detected among 147 patients was 12.9%.

The 35delG variant was one of the most commonly identified mutations in the different populations worldwide; however, studies carried out on patients from the Far East have reported a relatively low frequency or even the absence of this mutation, which is consistent with the Europe to Asia gradient in terms of the distribution of 35delG carriers. In several studies carried out in India and Korea, 35delG was not detected in any of the patients studied [25,26].

It was originally proposed that the high prevalence of 35delG might be because of a mutational hotspot [27]. However, later, Van Laer *et al.* [28] reported that this high frequency resulted from a founder effect, rather than from a mutational hotspot in *GJB2*.

A general rule in population genetics states that the geographic center of a mutation corresponds to the area where it is most frequent [29]. Among the current data, Greece, with a carrier frequency of 3.54% for the 35delG mutation, represents a probable focus. It has been suggested that gene flow of the 35delG mutation from Greece to the south of Italy could be explained by the ancient Greek colonization 'Magna Grecia'. This explains why the 35delG mutation is present with the highest frequencies in the focus (Greece), is at relatively elevated frequencies in the regions of ancient Greece colonization, and is comparatively less common elsewhere. Toward the southern Mediterranean coast, there are documented ancient Greek migrations in Tunisia, Morocco, Egypt, and Turkey. The actual patterns of the prevalence of 35delG around the Mediterranean Sea are interpreted in the light of these Greek colonizations [30].

All the patients in the present study homozygous for the 35delG showed a bilateral severe to profound SNHL. This observation is in agreement with other studies reviewed in the literature that reported that the 35delG homozygote state presents a high risk for severe hearing impairment [31]. An Egyptian study by Mohamed *et al.* [16] reported bilateral severe to profound SNHL in all the detected 35delG homozygous patients. In a Romanian study by Lazar *et al.* [32], all the 19 homozygous patients for the 35delG mutation had profound or severe hearing loss.

In the families in whom the deaf offspring were identified to be homozygous for the 35delG and the normal hearing parents were heterozygous carriers, they were informed that the other family members should consider undergoing carrier detection. These carriers were provided with genetic counseling to help them make informed decisions on family planning. According to the rules of the autosomal recessive inheritance pattern, these parents have a 25% recurrence chance of giving birth to another deaf child homozygous for the deletion, 50% probability of having a normal hearing child heterozygous for the deletion, and 25% probability of having a normal hearing child not carrying the deletion at all. All the offspring of the homozygous deaf patients will be carriers for the 35delG. The normal hearing carrier mothers can go through prenatal screening for the 35delG mutation detection or preimplantation genetic diagnosis because the deafness-causing mutation has been identified in

their deaf children. As both techniques are not widely requested in Egypt because of the religious beliefs, neonatal screening for the presence of the deletion (besides the audiological screening) can be established aiming for the early detection of SNHL. In one family, the normal hearing carrier maternal aunt was advised to avoid a consanguineous marriage. It is preferable for her future partner to go through screening for the 35delG mutation before marriage. In the other family, the normal hearing sib was reassured on the choice of his future partner and family plans as he was not a carrier for the deletion.

Families in whom the deaf offspring were identified to be heterozygous for the 35delG were advised to undergo testing with more sophisticated techniques for the detection of mutations other than 35delG or 167delT on the second allele. These techniques include direct DNA sequence analysis for whole exon two (the coding sequence) of the relatively small *GJB2* gene. Sequencing is considered an expensive and laborious technique, and although it will not be available in Egyptian diagnostic labs for several years, it will represent a major improvement.

In the current study, the 167delT mutation was not detected in any of the patients studied. However, few studies on the 167delT mutation frequency have been reported in various populations. To our knowledge, there have been no previous reports for the 167delT mutation in patients with nonsyndromic hearing impairment in Egypt. Several studies have been carried out in Turkey, Jordan, and Croatia in which 167delT was not detected in any of the deaf patients studied [17,18,33].

In an Israeli study by Lerer *et al.* [34], 27 patients with Jewish Ashkenazi origin were included. Twelve (44.4%) patients were homozygous for 167delT, two (7.4%) were homozygous for 35delG, and five (18.5%) were compound 167delT/35delG heterozygotes. Three (11%) patients in their study group were heterozygotes, two with 167delT and one with 35delG, with no other mutation identified on the other allele. In an Israeli study by Brownstein and Avraham [35], out of 230 Israeli Jewish patients, 21 (9.13%) of Ashkenazi origin were homozygotes for the 167delT mutation, 16 (6.9%) were homozygotes for the 35delG mutation, and 14 (6%) were compound heterozygotes for 35delG/167delT mutations. In an American study by Prasad *et al.* [36], the 167delT mutation was detected in 11 of 418 alleles, with an allele frequency of 2.63%. As such, screening for 167delT to identify *GJB2*-related deafness should be considered in populations with individuals of Ashkenazi Jewish descent. The conserved haplotype flanking 167delT suggests that this mutant

allele of *GJB2* is a founder mutation segregating in the Ashkenazi Jewish population [34,37].

In conclusion, the 35delG is a common pathogenic mutation and an important contributor toward ARNSHL in the Egyptian population, and it should be screened in any Egyptian individual with bilateral, prelingual, nonsyndromic SNHL, especially those with severe to profound degrees of deafness. The 167delT was not detected in any of the patients studied. In the patients with non-detected 35delG or 167delT, hearing impairment is genetically very heterogeneous; therefore, this result does not exclude other genetic causes of hearing impairment in these patients.

Acknowledgements

Conflicts of interest

None declared.

References

- Kral A, O'Donoghue GM. Profound deafness in childhood. *N Engl J Med* 2010; 363:1438–1450.
- Cordeiro-Silva Mde F, Barbosa A, Santiago M, Provetti M, Dettogni RS, Tovar TT, *et al.* Mutation analysis of *GJB2* and *GJB6* genes in Southeastern Brazilians with hereditary nonsyndromic deafness. *Mol Biol Rep* 2011; 38:1309–1313.
- Hilgert N, Smith RJ, Van Camp G. Forty-six genes causing nonsyndromic hearing impairment: which ones should be analyzed in DNA diagnostics? *Mutat Res* 2009; 681:189–196.
- Martinez AD, Acuna R, Figueroa V, Maripillan J, Nicholson B. Gap-junction channels dysfunction in deafness and hearing loss. *Antioxid Redox Signal* 2009; 11:309–322.
- Zhao HB, Kikuchi T, Ngezhahyo A, White TW. Gap junctions and cochlear homeostasis. *J Membr Biol* 2006; 209:177–186.
- Da Motta LH, Felix TM, de Souza LT, Lavinsky-Wolff M, Costa-Motta FM, de Faria MR, *et al.* Prevalence of the 35delG mutation in deaf South Brazilian infants submitted to cochlear implantation. *Int J Pediatr Otorhinolaryngol* 2012; 76:287–290.
- Radulesco L, Mărtu C, Birkenhager R, Cozma S, Ungureanu L, Laszig R. Prevalence of mutations located at the *dfnb1* locus in a population of cochlear implanted children in eastern Romania. *Int J Pediatr Otorhinolaryngol* 2012; 76:90–94.
- Smith RJ, Hone S. Genetic screening for deafness. *Pediatr Clin North Am* 2003; 50:315–329.
- Smith RJ. Clinical application of genetic testing for deafness. *Am J Med Genet* 2004; 130A:8–12.
- Storm K, Willocx S, Flothmann K, Van Camp G. Determination of the carrier frequency of the common *GJB2* (connexin-26) 35delG mutation in the Belgian population using an easy and reliable screening method. *Hum Mutat* 1999; 14:263–266.
- Bors A, Andrikovics H, Kalmar L, Erdei N, Galambos S, Losonczi A, *et al.* Frequencies of two common mutations (c.35delG and c.167delT) of the connexin 26 gene in different populations of Hungary. *Int J Mol Med* 2004; 14:1105–1108.
- Estivill X, Fortina P, Surrey S, Rabionet R, Melchionda S, D'Agruma L, *et al.* Connexin-26 mutations in sporadic and inherited sensorineural deafness. *Lancet* 1998; 351:394–398.
- Chan DK, Schrijver I, Chang KW. Diagnostic yield in the workup of congenital sensorineural hearing loss is dependent on patient ethnicity. *Otol Neurotol* 2011; 32:81–87.
- Snoeckx RL, Hassan DM, Kamal NM, Van Den Bogaert K, Van Camp G. Mutation analysis of the *GJB2* (connexin 26) gene in Egypt. *Hum Mutat* 2005; 26:60–61.
- Abdel-Meguid N, Omran MH, Dardir AA, Abdel-Raouf ER, Ghorab IA, *et al.* Study of 35delG in congenital sensorineural non-syndromic hearing loss in Egypt. *J Appl Sci Res* 2008; 4:621–626.
- Mohamed MR, Alesutan I, Föller M, Sopjani M, Bress A, Baur M, *et al.* Functional analysis of a novel 171N mutation in the *GJB2* gene among Southern Egyptians causing autosomal recessive hearing loss. *Cell Physiol Biochem* 2010; 26:959–966.
- Evirgen N, Solak M, Derekoy S, Erdogan M, Yildiz H, Eser B, *et al.* Genotyping for Cx26 and Cx30 mutations in cases with congenital hearing loss. *Genet Test* 2008; 12:253–256.
- Mahasneh AA, Battah RM. Prevalence of connexin 26 mutations in patients from Jordan with non syndromic hearing loss. *Int J Hum Genet* 2006; 6:119–124.
- Shahin H, Walsh T, Sobe T, Lynch E, King MC, Avraham KB, *et al.* Genetics of congenital deafness in the Palestinian population: multiple connexin 26 alleles with shared origins in the Middle East. *Hum Genet* 2002; 110:284–289.
- Gazzaz B, Weil D, Rais L, Akhyat O, Azeddoug H, Nadifi S. Autosomal recessive and sporadic deafness in Morocco: high frequency of the 35delG *GJB2* mutation and absence of the 342-kb *GJB6* variant. *Hear Res* 2005; 210:80–84.
- Trabelsi M, Bahri W, Habibi M, Zainine R, Maazoul F, Ghazi B, *et al.* *GJB2* and *GJB6* screening in Tunisian patients with autosomal recessive deafness. *Int J Pediatr Otorhinolaryngol* 2013; 77:714–716.
- Pampanos A, Economides J, Iliadou V, Neou P, Leotsakos P, Voyiatzis N, *et al.* Prevalence of *GJB2* mutations in prelingual deafness in the Greek population. *Int J Pediatr Otorhinolaryngol* 2002; 65:101–108.
- 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.
- Gabriel H, Kupsch P, Sudendey J, Winterhager E, Jahnke K, Lautermann J. Mutations in the connexin26/*GJB2* gene are the most common event in non-syndromic hearing loss among the German population. *Hum Mutat* 2001; 17:521–522.
- Joseph AY, Rasool TJ. 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.
- Lee KY, Choi SY, Bae JW, Kim S, Chung KW, Drayna D, *et al.* Molecular analysis of the *GJB2*, *GJB6* and *SLC26A4* genes in Korean deafness patients. *Int J Pediatr Otorhinolaryngol* 2008; 72:1301–1309.
- Denoyelle F, Weil D, Maw MA, Wilcox SA, Lench NJ, Allen-Powell DR, *et al.* Prelingual deafness: high prevalence of a 30delG mutation in the connexin 26 gene. *Hum Mol Genet* 1997; 6:2173–2177.
- Van Laer L, Coucke P, Mueller RF, Caethoven G, Flothmann K, Prasad SD, *et al.* A common founder for the 35delG *GJB2* gene mutation in connexin 26 hearing impairment. *J Med Genet* 2001; 38:515–518.
- Watterson GA, Guess HA. Is the most frequent allele the oldest? *Theor Popul Biol* 1977; 11:141–160.
- Lucotte G. High prevalences of carriers of the 35delG mutation of connexin 26 in the Mediterranean area. *Int J Pediatr Otorhinolaryngol* 2007; 71:741–746.
- Janecke AR, Hirst-Stadlmann A, Günther B, Utermann B, Müller T, Löffler J, *et al.* Progressive hearing loss, and recurrent sudden sensorineural hearing loss associated with *GJB2* mutations — phenotypic spectrum and frequencies of *GJB2* mutations in Austria. *Hum Genet* 2002; 111:145–153.
- Lazar C, Popp R, Trifa A, Mocanu C, Mihut G, Al-Khrouz C, *et al.* Prevalence of the c.35delG and p.W24X mutations in the *GJB2* gene in patients with nonsyndromic hearing loss from North–West Romania. *Int J Pediatr Otorhinolaryngol* 2010; 74:351–355.
- Sansovic I, Knezevic J, Musani V, Seeman P, Barisic I, Pavelic J. *GJB2* mutations in patients with nonsyndromic hearing loss from Croatia. *Genet Test Mol Biomarkers* 2009; 13:693–699.
- Lerer I, Sagi M, Malamud E, Levi H, Raas-Rothschild A, Abeliovich D. Contribution of connexin 26 mutations to nonsyndromic deafness in Ashkenazi patients and the variable phenotypic effect of the mutation 167delT. *Am J Med Genet* 2000; 95:53–56.
- Brownstein Z, Avraham KB. Deafness genes in Israel: implications for diagnostics in the clinic. *Pediatr Res* 2009; 66:128–134.
- Prasad S, Cucci RA, Green GE, Smith RJ. Genetic testing for hereditary hearing loss: connexin 26 (*GJB2*) allele variants and two novel deafness-causing mutations (R32C and 645–648delTAGA). *Hum Mutat* 2000; 16:502–508.
- Morell RJ, Kim HJ, Hood LJ, Goforth L, Friderici K, Fisher R, *et al.* Mutations in the connexin 26 gene (*GJB2*) among Ashkenazi Jews with nonsyndromic recessive deafness. *N Engl J Med* 1998; 339:1500–1505.