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

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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

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 β2 (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.
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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 DNA Blood 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 BslI 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]

<table>
<thead>
<tr>
<th>Mutation</th>
<th>PCR product (bp)</th>
<th>Restriction enzyme</th>
<th>Digest homozygous normal (bp)</th>
<th>Digest homozygous Mutant (bp)</th>
<th>Digest heterozygous carrier (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>35delG</td>
<td>207</td>
<td>BslI</td>
<td>207</td>
<td>181,26</td>
<td>207,181,26</td>
</tr>
</tbody>
</table>
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.

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 families in whom the deaf offspring were identified to be homozygous 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