BJMG 14/1 (2011) 19-24
10.2478/v10034-011-0013-4

ORIGINAL ARTICLE

DETECTION OF THE GJB2 MUTATION IN IRANIAN CHILDREN WITH HEARING LOSS TREATED WITH COCHLEAR IMPLANTATION

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ABSTRACT

The 35delG mutation in the gap junction protein, β2, 26kDa (*GJB2*) gene is the most common mutation that has been found in children with non syndromic hearing loss. Testing for the *GJB2* gene mutation is simple and can directly answer the concerns of the parents about cause of the disorder and prognosis for their children. Cochlear implantation (CI) is one of the methods of hearing rehabilitation in patients with complete hearing loss. The present study was designed for genetic assessment of children who were referred for CI.

Connexin 26 (Cx26) gene analyses were performed on 42 children with non syndromic hearing loss who were referred to the Baqiyatallah Hospital, Tehran, Iran for genetic consultation and CI. Clinical history was obtained and an examination conducted on each individual. Genomic DNA was extracted from peripheral blood and mutation identification of the Cx26 gene was performed by polymerase chain reaction (PCR) amplification and direct sequencing of the coding sequence of the gene. Cochlear implantation was performed for all patients and treatment response was assessed for all of them based on speech intelligibility rating (SIR) before and after CI.

We found six patients (14.3%) with the 35delG mutation on the Cx26 gene, two homozygotes and four heterozygotes. No other mutation was detected. Treatment response in children with the homozygous 35delG mutation was better than in heterozygous patients, and treatment response in children with the mutation was better than in children with no mutation.

Mutation screening for finding deafness causing mutations in the *GJB*2 gene is a useful predictor of post-implantation speech perception. We suggest microarray or other advanced mutation detection methods for assessment of other genes that might be responsible for non syndromic deafness.

Keywords: Gap junction protein, β2, 26kDa (*GJB2*) gene mutation; Hearing loss; Cochlear implantation (CI); Connexin 26 (Cx26) gene

INTRODUCTION

In genetic assessment of hearing impairment, 46 genes and 81 genetic loci have now been identified and these numbers are increasing [1]. The 35delG mutation on the gap junction protein, β2, 26kDa (*GJB2*) gene is the most common mutation to be found in children

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with non syndromic hearing loss and has been identified as the predominant cause of inherited sensorineural deafness [2-7]. The *GJB2* gene encodes gap junction channel protein 26 [connexin 26 (Cx26)] which allows direct communication between the cells [8,9]. The Cx26 gene is one member of a family of related gap-junction channel-forming proteins, each of which is commonly named from its molecular weight (Cx26, Cx30, *etc.*). The genes for 20 different connexin proteins are present in the human genome [9]. Since 1990, genetic assessment of the GJB2 mutation has been performed in children with congenital hearing loss. Now, the American College of Medical Genetics (Bethesda, MD, USA) has presented genetic testing as a routine method for the evaluation of children with congenital hearing loss [10].

Between 27 to 39% of deaf children show abnormalities such as cochlear dysplasia, lateral semicircular canal dysplasia and dilated vestibular aqueduct on imaging studies [computed tomography (CT) and magnetic resonance imaging (MRI)] [11,12]. Imaging studies are expensive and cannot suitably address the cause and prognosis of hearing loss in these children and thus cannot be accepted as the first method of choice. On the other hand, GJB2 testing is simple and can give direct answers to the parents' concern about the cause and prognosis in their children.

Cochlear implantation (CI) is one of the methods of hearing rehabilitation in patients with complete hearing loss and nowadays most children with dominant or recessive congenital hearing loss are candidates for this therapeutic method [13]. The present study had two main purposes: firstly, genetic assessment of children who were referred for CI, and secondly, to determine whether the type of mutation is an important factor in treatment of the studied children.

PATIENTS AND METHODS

Study Subjects. Connexin 26 gene analysis was performed on 42 children (20 males and 22 females, age range 4-12 years old, average 6 years old) with non syndromic hearing loss and with normal parents who were referred to Baqiyatallah Hospital, Tehran, Iran, for genetic consultation and CI. Clinical history was obtained and an examination conducted on each individual, with special emphasis on identifying potential environmental causes of hearing loss, such as infections, trauma, exposure to known or possible ototoxic drugs and looking for evidence of syndromic forms of deafness. All subjects were otoscopically examined and pure tone audiometry was performed on all subjects. Air conduction thresholds were measured at 250Hz, 500Hz, 1kHz, 2kHz, 4kHz, 6kHz and 8kHz. All probands had prelingual deafness (early-onset).

Mutation Analysis. Genomic DNA was extracted from peripheral blood using the standard phenol-chloroform methods [14]. For mutation identification in the Cx26 gene, the coding sequence of the gene was polymerase chain reaction (PCR) amplified using primers Conn-F (5'-CTC CCT GTT CTG TCC TAG CT-3') and Conn-R (5'-CTC ATC CCT CTC ATG CTG TC-5'). The PCR conditions were 95°C for 3 min., then 32 cycles at 94°C, 59°C and 72°C, each for 45 seconds, followed by a 7 min. extension at 72°C. The PCR products were purified on agarose gel and directly sequenced using the same forward and reverse primers.

Treatment Response Assessment. Cochlear implantation was performed on all patients. Treatment response was assessed in all of them based on their speech intelligibility rating (SIR) before, and 3, 6 and 12 months after CI.

RESULTS

Mutation Detection in Our Subjects and Their Response to Cochlear Implantation. In the present study we analyzed the coding region of the Cx26 gene in 42 children with non syndromic hearing loss and found two homozygotes and four heterozygotes for the 35delG mutation (Figure 1). In other words, six patients (14.3%) had the 35delG mutation on the Cx26

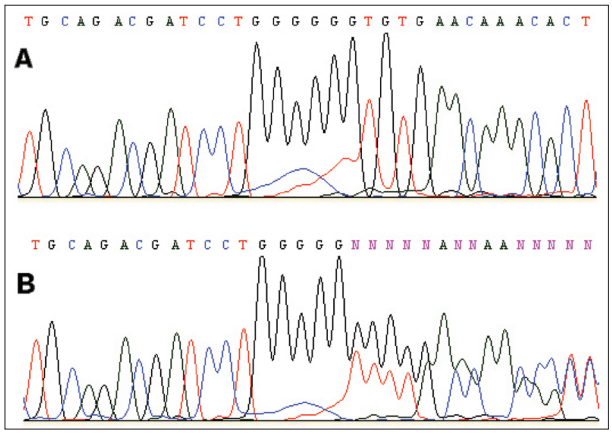

Figure 1. A part of the coding sequence of the Cx26 gene in a patient without (A) and a patient with the heterozygous 35delG mutation (B).

gene. No other mutations were identified in our subjects. Mutation frequency in the patients is shown in Figure 2.

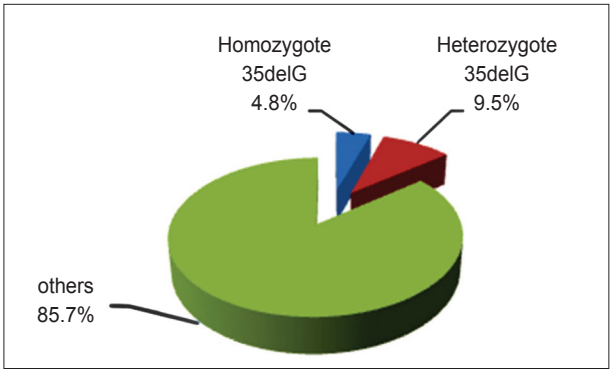

Figure 2. Mutation frequency in the patients.

Treatment response was assessed in all patients. According to results of SIR, it seems that treatment response in children with the mutation was better than in children with no mutation, and also treatment response in children homozygous for the 35delG mutation was better than in heterozygous patients (Figures 3 and 4).

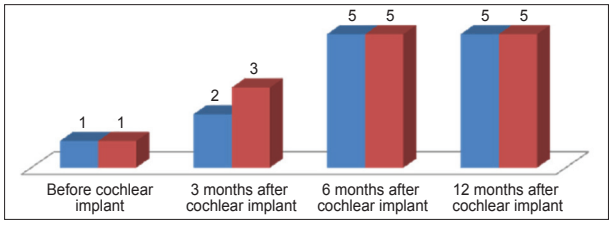

Figure 3. Speech intelligibility rating in patients with the homozygous 35delG mutation in Cx26.

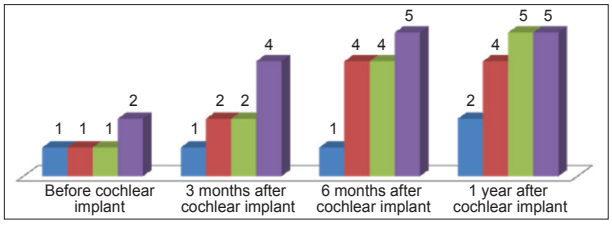

Figure 4. Speech intelligibility rating in patients with the heterozygous 35delG mutation in Cx26.

Details of SIR scores of included children before and 3, 6 and 12 months after CI are shown in Table 1.

DISCUSSION

The 35delG mutation accounts for approximately 70% of *GJB2* mutant alleles in Northern and Southern European, as well as American Caucasian populations, with a carrier frequency of 2-4%. In the Iranian population this mutation accounts for approximately 12-21% of all involved mutations in autosomal recessive non syndromic hearing loss [14-18]. In our study, the Cx26 genetic defect was seen in 14.3% of the investigated children with hearing impairment. Deafness in our patients had an autosomal recessive inheritance pattern and according to this pattern, we expected to see deafness only in children with homozygous mutations but in four patients, the mutations were heterozygous. In their study, del Castillo *et al.* (19) suggested that other mutations are present in a gene or genes in the same chromosomal region. This phenomenon could be explained on the basis of either a monogenic or a digenic pattern of inheritance. In the case of a monogenic mode of inheritance, there must be a regulatory element that is essential for the expression of the *GJB2* gene in the inner ear. This hypothetical element would be located far upstream of GJB2, and the deletion of this element would suppress the level of expression of the *GJB2* gene enough to produce a phenotype of hearing impairment. An alternative interpretation would be that the deletion inactivates a second gene whose protein is functionally related to Cx26.

Inheritance pattern by single or double genes is responsible for this phenomenon. In our mutation assessment of the Cx26 gene, the 35delG mutation was more frequent, while in Ashkenazi Jewish subjects, the 167delT mutation was more frequent [20].

Fukushima *et al.* [21] in their study on seven Japanese deaf children reported that better speech performance after CI may be observed in persons with *GJB2*-related deafness compared to children without *GJB2*-related deafness. Some authors such as Jun *et al.* [22] based on the microscopic evaluation of temporal histopathology in deaf children with GJB2 mutations concluded that a greater number of functional cells in the spiral ganglion of these children could explain their better speech performance. The GJB2 deafness phenotype is considered as typical non syndromic hearing loss and differences in their response to CI might be due to differences in higher brain function abilities [21].

Table 1. Average speech intelligibility rating before and after cochlear implementation in the children according to their mutation situation.

Patients		**Average Speech Intelligibility Rating**		
	Before CI	3 months later	6 months later	12 months later
36 children without mutations	1.06	1.44	2.26	3.11
2 homozygous children	1.00	2.50	5.00	5.00
4 heterozygous children	1.25	2.25	3.50	4.00

CONCLUSIONS

The results of our study showed that CI is a suitable choice for treatment of children with non syndromic deafness, especially with mutations in the *GJB2* gene. Mutation screening for deafness-causing mutations in the *GJB*2 gene is a useful predictor of post-implantation speech perception and permits better pre-implantation counseling. We suggest microarray or other advanced mutation detection methods for assessment of other genes which might be responsible for non syndromic deafness.

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