Int. Adv. Otol. 2011; 7:(3) 361-371

ORIGINAL ARTICLE

GJB2 35delG and Mitochondrial A1555G Mutations and Etiology of Deafness at the Gelibolu School for the Deaf in Turkey

Fatma Silan, Oguz Guclu, Laliz Esin Kadioglu, Coskun Silan, Sinem Atik, Ahmet Uludag, Asli Demiray, Ozturk Ozdemir, Fevzi Sefa Derekoy

Department of Medical Genetics (FS, SA, AU, OO)
Department of Otorhinolaryngology (OG, FSD)
Department of Paediatrics (LEK)
Department of Pharmacology (CS)
Audiometrist, Çanakkale Onsekiz Mart University School of Medicine, Çanakkale Turkey (AD)

Objective: 35delG mutation in the GJB2 (gap junction protein beta 2, connexin 26) gene is the most frequent mutation in patients with non-syndromic autosomal recessive deafness. The A1555G mutation in the mitochondrial 12S rRNA is another important genetic alteration, and is associated with aminoglycoside-induced deafness. The aim of this study was to explore the etiology of deafness and the prevalence of both mutations in the study cases.

Materials and Methods: We examined audiological and dysmorphological features of all children at the Gelibolu School for the deaf. A questionnaire investigating prenatal, perinatal and postnatal etiological causes of deafness was prepared, and pedigree analysis was performed for each individual. After ENT examination, audiological tests and mutation analysis with the RT PCR method were carried out.

Results: The GJB2 35delG and mitochondrial A1555G mutations were detected in 12% and 10% of all deaf school children, respectively. The percentages of genetic, acquired, both genetic and environmental, and unknown etiologies were 62.5, 20.3, 15.6 and 1.6, respectively. One patient had both Waardenburg Syndrome and the mitochondrial A1555G mutation, and one patient carried both 35delG and mitochondrial A1555G mutations. Interestingly, one sporadic case, who developed deafness after fever and aminoglycoside treatment, was found to have a homozygous 35delG mutation. His parents and healthy brother were heterozygous for the mutation.

Discussion: Our results showed that dysmorphologic examination and mutation analysis are important for the clarification of etiology, and that they can be helpful for genetic counselling.

Submitted : 21 September 2010 *Revised: 17 January 2011* *Accepted : 31 March 2011*

Introduction

Prelingual sensorineural hearing loss is the most common sensorial disorder in children with a birth prevalence of 1/650 [1]. A genetic basis accounts for about 40-60% of deafness cases, and 70-80% of these cases are nonsyndromic. Most of the nonsyndromic genetic prelingual SNHL cases show autosomal recessive (77-87%), autosomal dominant (8-22%), X-linked (1-5%) or mitochondrial (1%) inheritance [2,3].

Mutations in GJB2 are the most common cause of autosomal recessive nonsyndromic deafness in many populations worldwide [4]. 35delG mutation, which is the most frequent GJB2 mutation, accounts for 48-77% of all GJB2 mutations [5,6]. Since children with GJB2-related deafness show greater improvement in treatments with cochlear implants than children with GJB2-unrelated deafness, mutation analysis provides useful prognostic information. The mutation analysis can be helpful for the selection of appropriate treatment and accurate estimation of the etiology. It can also provide helpful information for genetic counselling [7-11].

Corresponding address:
Fatma Silan
Department of Medical Genetics Çanakkale Onsekiz Mart University School of Medicine,
17100 Çanakkale Turkey
Phone: 0090 286 2635950'1080 • Fax: 0090 286 2635957
E-mail: fsilan@yahoo.com

Mitochondrial 12S ribosomal RNA is primary target of aminoglycosides, as it is structurally similar to bacterial ribosomal RNA. Mitochondrial A1555G mutation is responsible for both aminoglycoside-induced and nonsyndromic hearing loss in many families worldwide [12]. Previous studies reported that diseases with high fever and febrile convulsions are the most common acquired etiological factors in Turkey [3,13]. Usage of toxic drugs in sick infants with fever may cause deafness, and mitochondrial A1555G mutation can also be a risk factor [14].

In this study we aimed to clearly determine the etiology of deafness, to minimize the number of the unknown etiological groups and analyze significance of GJB2 35delG and mitochondrial A1555G mutations on development of disease in Turkish deaf children.

Materials and Methods

Students enrolled in the Gelibolu School for the deaf for the 2009-2010 academic year were included in the study. Medical and family histories of the patients were examined by means of a comprehensive questionnaire filled out by the children and their teachers. To validate the obtained data, detailed information was obtained from the parents about pregnancy, labour and postnatal diseases, as well as consanguinity status and family history of deafness and/or syndromic symptoms. Although the parents of 21 probands were not accessible, parents of 43 probands were contacted. All children were examined by the same medical geneticist for identification of syndromic etiology.

The same physician and audiometrist performed otologic and audiologic examinations in all cases. Deformities of auricular canal, external canal and tympanic membrane were evaluated. The audiologic examination included a pure tone audiogram (İnteracoustics AC40, Denmark), tympanometry and acoustic reflex (Interacoustics AZ26, Denmark), otoacoustic emmissions and auiditory brainstem responses (ABR) (Interacoustics Eclipse, Denmark). The degree of hearing loss (best ear, average 500-2000 Hz) was recorded as mild (15-30 dB), moderate (31-60dB), severe (61-90 dB), profound (90-120 dB) or total ≥121.

ABR and transient evoked otoacoustic emissions (TEOAE) were measured for all cases in order to determine auditory neuropathy. Any sedative drug was not administered to the samples before the examination. Tests were performed in a silent room when the samples were immobile in a sleeping situation. During ABR measurements, the samples were exposed to 100 and 90 dB SPL, initially. If a threshold at 90 dB SPL was detected, measurements at lower levels were also performed. TEOAE measurements were repeated at least twice. We evaluated the records as pass, fail or borderline. The pass criterium was hearing the Signal Noise Ratio(SNR)≥3dB at three frequencies between 1 – 4 kHz band noise. The borderline criterium was SNR≥3dB at two frequencies.

We grouped the cases according to the etiological features: Genetic, environmental, genetic and environmental and unknown. If other hearing loss cases were observed within the family (familial cases), any mutation was detected or another syndrome associated with hearing loss was identified, those cases were categorized under the genetic etiology group. The cases were categorized under the acquired group if any factors such as prenatal infections, low birth weight, prematurity and postnatal infections were stated in anamnesis and patient records. Consanguineous marriages were not directly considered to be etiological evidences, but they are taken into account for the determination of inheritance pattern. Consanguinity statuses of parents were categorized into four groups: first-degree consanguinity, distant consanguinity, being from the same village and non-consanguinity. The inheritance patterns of the cases were categorized according the following criteria: autosomal dominant inheritance (AD) if one of the parents had hearing loss, autosomal recessive inheritance if siblings and/or cousins also had hearing loss or if 35delG mutation was identified, X-linked inheritance if only male members of the family had hearing loss and the males in the pedigree were linked through unaffected females, or mitochondrial inheritance if mitochondrial A1555G mutation was identified.

Genomic DNA was extracted from peripheral blood samples. Blood drawn from the antecubital vein was immediately transferred into a sterile EDTA containing tube. Total genomic DNA was extracted from peripheral blood samples by the Magna Pure Compact (Roche) and stored at -20˚C until genetic analysis. The genomic DNAs of all samples were analyzed for 2

gene mutations (GJB2 35delG, mitochondrial A1555G). Two mutations were analyzed with Real Time PCR and LightCycler 2.0 Roche. The wild type, heterozygous and homozygous mutant genotype profiles of the genes were determined according to the obtained Tm values of each gene (Figure 1).

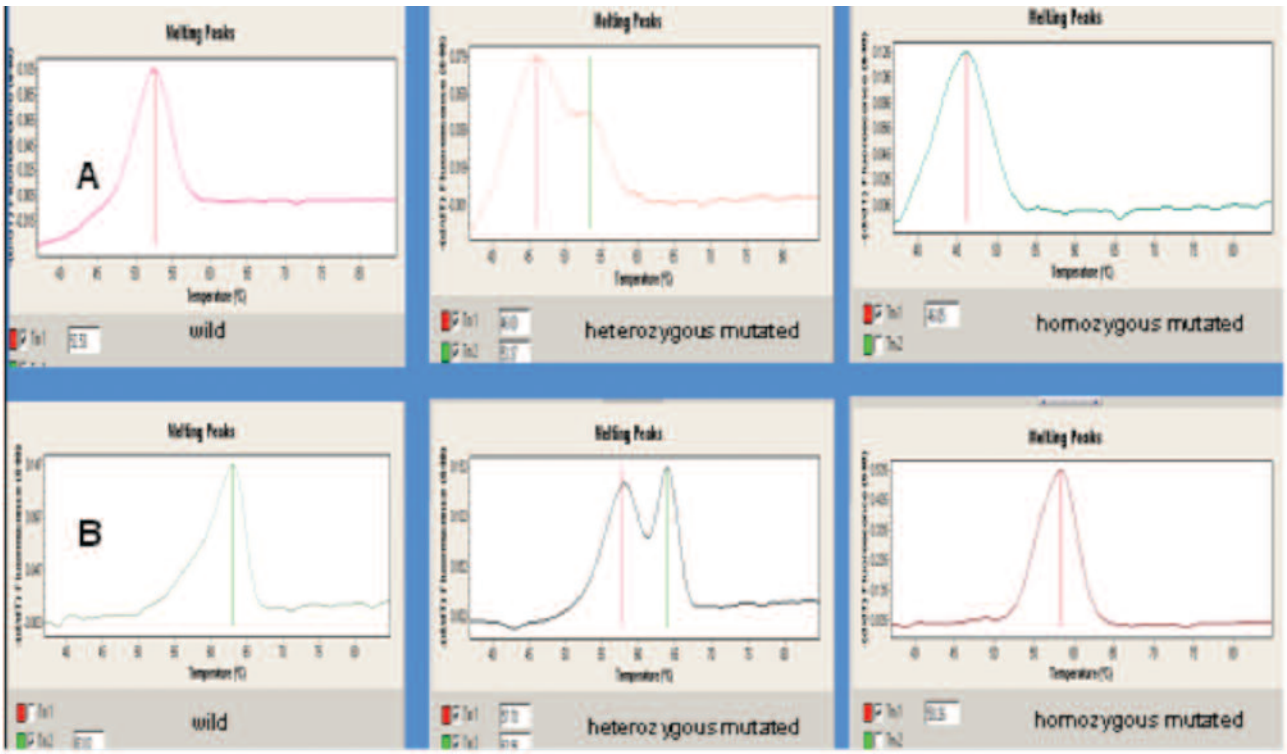

Figure 1. The figure shows Wild and Mutated profiles of Connexin 26 35delG and Mitochondrial A1555G genes at Real Time PCR

Results

The average age of the 64 patients included in this study was 16.78. Five patients were female whereas the remaining were male. Such a low female student number stemmed from the situation that the school was a regional boarding school for boys, and only the female students living near the school could attend.

The otological examination revealed that the most frequent auricular anomaly was protruding auricle (9.4%). Additionally, asymmetric auricle sizes (4.6%), tragal deformations (1.5%) and pre-auricular sinus (1.5%) were observed. Otoscopy revealed no external canal anomalies, but did show tympanic membrane retractions (7.8%) and myringosclerosis (4.6%). Pure tone odometry results revealed that 15 patients (23.4%) had total hearing loss, 44 patients had profound hearing loss (68.8%), and 5 patients (7.8%) had severe hearing loss in their left ears. On the other hand, one patient (29.7%) had total, 38 patients had profound (59.3%) and 7 patients had severe (10.9%) hearing loss (Table 1). A type A tympanogram was obtained in all cases. Acoustic reflex were obtained bilaterally on 10 patients (15.6%). Six of them had severe and four of them had profound or total hearing loss. ABR thresholds could be obtained from the left ear of 15 patients (23.4%) and from the right ear of patients 20 (31.3%). None of the patients passed the TEOAE test or had borderline emissions. Therefore, none of them was considered to have auditory neuropathy.

Table 1. Hearing loss levels of children

Hearing loss level	Left Ear	Right Ear
Severe	5(7.8%)	7(10.9%)
Profound	44(68.8%)	38(59.3%)
Total	15(23.4%)	19(29.8%)

Patients were classified into four main groups according to the etiology of their hearing loss: genetic, environmental, both genetic and environmental, and unknown etiology. Forty patients had genetic (62.5%), 10 patients had both genetic and environmental (15.6%), 13 patients had environmental (20.3%) and 1 patient had an unknown etiology (1.6%) (Table 2). Eighteen of the genetic cases were sporadic whereas 31 of them were familial cases. Sporadic cases were classified as genetic because either they were syndromic or their patients were found to have mutations. Ten of the sporadic cases were syndromic: three patients were homozygous and three patients were heterozygous for 35delG/GJB whereas one patient had both GJB 35delG and mitochondrial A1555G mutations and one patient had only mitochondrial A1555G mutation. If the mutation analysis had not been performed, eight cases would have been classified has having an unknown etiology. Among these cases, parents of four mutation-containing cases (1 case with homozygous 35delG, 1 case with heterozygous 35delG, 2 cases with mitochondrial mutation) reported high fever and/or febrile convulsion history within the first postnatal year. Parents of the case with homozygous 35delG stated that he could respond to sounds and did not exhibit any sign of hearing problems prior to the fever-causing disease. Parents of the case with homozygous 35delG stated that he could respond to sounds and did not exhibit any sign of hearing problems prior to the disease with high fever when he was six months old. They stated that he had hearing loss after this disease with high fever and a third aminoglycoside dose. The parents and healthy sibling of the proband were also found to be heterozygous for the mutation. Twenty percent of the cases with genetic etiology (10/49) and 15% of all cases (10/65) were syndromic (Table 3). Fifteen of all cases were syndromic cases: 2 cases of (sporadic) Waardenburg Type 1, 5 cases of (4 sporadic and 1 familial) Waardenburg Type 2, 1 case of (familial) Usher Type 1, 1 case of (sporadic) otooculodigital syndrome, 1 case of (sporadic) Pendred syndrome, 1 case of (sporadic) deafness and obesity, 1 case of (sporadic) hypopigmentation / deafness and 3 cases of (sporadic) unclassified syndromes (Table 5).

Table 2. Etiologic classification.

	Number of cases	Percentage (%)
Genetic	40	62,5
Both Genetic and Acquired	10	15,6
Acquired	13	20,3
Unknown	1	1,6

Table 3. Genetic etiology classification

	Familial	Sporadic	Total(%all case, %genetic)
Syndromic	5	10	15 (23, 37,5)
	AR, AD, X, M	AR, M	Total(%all case, %genetic)
Nonsyndromic	20, 2, 2, 3	5, 3	35 (55, 70)

Table 4. Classification of consanguineous marriages

	Consanguineous marriages			
	First degree	2nd/3rd degree	Same village	Unknown
Genetic	12	5	6	7
Non-genetic	2	1	1	8

Table 5. Syndromes

Name of the Syndrome	Number of Cases	Sporadic (S), Familial (F)
Waardenburg Type 1	2	2S
Waardenburg Type 2	5	1S, 4F
Usher Type 1	1	F
Otooculodigital syndrome	1	S
Pendred	1	S
Choroideremia with deafness and obesity	1	S
Hypopigmentation/deafness	1	S
Unidentified digital syndrome	3	3S
Total	15	10.5

Consanguineous marriages were divided into four groups: first-degree consanguinity, second or third degree consanguinity, from the same village or of unknown status. Out of the parents of 12 probands with a genetic etiology, 5 cases were first degree relatives, 2 cases were second or third degree relatives, 6 cases were from the same village and the consanguinity status of 7 marriages were unknown. Among the cases with a non-genetic etiology, 2 families were a first-degree consanguineous marriage, 1 family had cases of second- or third-degree consanguineous marriage, one family was from the same village and the consanguinity statuses of eight families were unknown (Table 4).

The etiology of the acquired deafness was divided into 7 groups: febrile convulsion, meningitis, diseases with high fever, birth trauma/asphyxia, prematurity, low birth weight, ototoxicity (high fever history). 3 children had had febrile convulsions, 2 had suffered meningitis, 11 had had diseases with high fever (3 having also ototoxicity story), 3 had undergone birth trauma/asphyxia, 3 suffered prematurity and 1 had a low birth weight story (Table 6).

Out of 64 cases, 8 cases had GJB2 35delG mutations. Three of them were heterozygous while 5 of them were homozygous for the mutation. Seven cases had heterozygous mitochondrial A1555G mutations (Table 7).

Table 6. Acquired deafness reasons

Name of Disease	Environmental	Genetic+ Environmental
Febrile convulsion	-	3
Meningitis	1	1
Diseases with high fever	6	5 (3*)
Birth trauma/asphyxia	3	-
Prematurity	2	1
Low birth weight	1	-
Ototoxicity (with high fever)		3*
Total:	13	10

**: Ototoxicity story*

Table 7. Mutation types identified in patients

Mutation type	Connexin 26 35 delG (GJB2) (n/%)	Mitochondrial (n/%)
Wild	56	57
Heterozygous	3	7
Homozygous	5	0

2 of 7 cases with A1555G mutation were sporadic, and ototoxicity associated with aminoglycoside usage in treatment of high fever was identified. Out of these 7 cases, 3 had familial nonsyndromic, 1 had sporadic nonsyndromic and 1 had familial syndromic (WS) etiology.

That is to say, out of 7 cases with A155G mutation, 4 were familial and 3 were sporadic, whereas out of 8 cases with 35delG mutation, 2 were familial and 6 were sporadic cases.

Of 8 cases with 35delG mutation, 5 were homozygous and 3 were heterozygous. In addition, one of the heterozygote patients for the 35delG mutation also carried mitochondrial A1555G. Among these cases, only 2 cases with homozygous mutation exhibited familial nonsyndromic hearing loss. Six of the cases were sporadic, and 4 of them had a high fever history. Of the cases with high fever history, 2 were homozygous and 1 was heterozygous for the 35delG mutation, whereas 1 was carrying 35delG/A1555G mutations.

Discussion

Deafness is an important health problem interfering with communication and learning capacity. Since audition is crucial for proper development, greater emphasis should be laid on hearing problems. Since hearing screening tests for infants are routinely applied in Turkey, our opportunity to identify hearing loss cases in the first six months has increased. Detection of families at genetic risk for hearing loss will provide an earlier diagnosis [15,16].

Pathologies of the auricle, external canal and tympanic membrane were evaluated during physical examination. The protruding auricle was the most frequent auricle deformity that we observed. The prevalence of this deformity in the general population was defined as 5% in literature [17]. Our proportion is high when compared to this one. Asymmetric auricle size, tragal deformity and pre-auricular sinus were the other anomalies identified. We did not observe any cases of external auditory canal atresia. The most frequent tympanic membrane problem was retracting the tympanic membrane. Although we observed myringosclerosis cases, more complex pathologies, such as retraction pouch, perforation, and otitis media with effusion, adhesive otitits and atelectasis were not detected. Tympanometry results were pointed out on a type A tympanogram.

Most patients suffered from profound or total hearing loss. This situation probably stems from the fact that this study was conducted at a school for the deaf. In Turkey, children with better hearing skills generally attend regular schools after a rehabilitation period. In the study of Derekoy cases in a school for the deaf in Afyon, 93% of patients were defined to have profound hearing loss (13). Silan et al. performed a hearing test in 443 cases, and they identified 256 profound or total and 175 severe hearing loss cases [18].

Auditory neuropathy (AN) is a pathology where outer hairy cells function normally but the transmission of sound from inner hairy cells to eight cranial nerves is impaired. Therefore, the patient has normal otoacouistic emissions, but auditory brainstem responses are abnormal or absent. The incidence of AN among the neonates with a high risk for hearing impairment was reported between 0.23% and 1.3% in literature [19]. It was also reported that this ratio was 1.8-14% among the children who suffer from severe hearing loss. Duman et al. reported 4% AN among the deaf school students in Turkey [19]. However we did not detect any AN cases in this study. The actual incidence of this rarely observed pathology will be determined after obtaining results of national screening programs. In addition to OAE, an ABR test should also be performed during the infant hearing screening to identify this pathology.

Genetic and environmental factors are the main etiology underlying deafness in Turkey [3]. Identification of an environmental factor generally keeps doctors and researchers from exploring the genetic etiology of the disease, and it is even accepted as exclusion criterion [3,20]. In our study, regardless of any preliminary criteria, all cases were analyzed in terms of environmental etiology, syndromic etiology, and family history, and carrying GJB 235delG and mitochondrial A1555G mutations. Moreover, some cases were identified as being affected by more than one genetic factor (e.g. coexistence of Waardenburg Syndrome and mitochondrial mutation). This information may be taken under consideration by genetic counselors so as not to miss any risk factors. This finding can explain why some members suffer from hearing loss, whereas others within the same family do not, for some diseases such as Waardenburg syndrome, which exhibits differential expressivity [21]. Nye et al. reported mitochondrial A1555G mutation in

a case that included Waardenburg syndrome, deafness and neural tube defect [22]. Our case is the second to be reported in the literature, which verifies that the case is not just a coincidence.

Congenital (white forelock, iris heterochromia, cleft lip-palate, polydactyl) or acquired (thyroid anomalies, retinitis pigmentosa) symptoms accompanied by hearing loss point out a syndromic and genetic etiology even in the absence of other cases [19,23]. Syndromic etiology was identified in 23% of our cases while genetic etiology underlies 37% of our cases. Waardenburg is one of the most frequent syndromes to be accompanied by hearing loss. It was the most frequent syndrome in our previous study conducted among school-age children, and the second most frequent one in the study completed by Tekin et al. [15]. In some studies in which Usher syndrome was detected to be the most frequent cause for hearing loss, patients developed vision loss during or after adolescence [24]. Our cases might not have developed vision loss yet they were being school-age children. Additionally, since Usher syndrome type 1 results in a developmental delay in walking, these children may not be able to attend school. Many studies including that of Riga et al. showed that the syndromic etiology of deafness cases, as applied to those studied at the hospital, was different from that of the cases studied at schools for the deaf [25]. It is normal that syndromes accompanied by intellectual disability are not observed among school-age children. Moreover, children with severe visual and/or balance problems and severe extremity anomalies cannot attend school for the deaf. In Turkey, only severely and profoundly deaf children are accepted to the schools for the deaf. Mildly and moderately deaf children are educated at regular schools after obtaining a certain amount of recovery following amplification treatment. Silan et al. stated that children attending a school where mild to moderate deafness cases predominated (Halıcıoğlu Primary School) usually had several various syndromes, although the authors did not perform a clinical examination for each child [18]. In addition, they published the data together with data collected from other schools. Children with severe cerebral and cardiac anomalies may die before a diagnosis of deafness. Syndromic cases probably account for a greater portion of hearing loss cases. Waardenburg, Usher and Pendred syndromes, as well as Jervel Lange Nielsen syndrome (which was not observed among our cases), should still be considered to be the most frequent syndromes. Patients with Waardenburg syndrome benefit more from cochlear implants compared to sufferers of other syndromes. On the other hand, early implantation is effective in Usher syndrome and implantation should be performed before the onset of vision loss [24,26].

Only 5 of our cases were female, whereas the remaining cases were male. At first glance, this situation gives rise to the thought that X-linked cases account for an important portion of etiology. However, this probably stems from the fact that this school was a regional boarding school for boys, and only the female students accommodated near the school could attend the school as day students. Only 2 of our cases were clearly diagnosed as having X-linked inheritance.

Two of our 7 patients with mitochondrial A1555G mutation were sporadic, and ototoxicity associated with aminoglycoside usage against high fever was a possibility. Out of these 7 cases, 3 had familial nonsyndromic, 1 had sporadic nonsyndromic and 1 had familial syndromic (WS) etiology. Thanks to this mutation analysis, 2 cases thought to be purely acquired cases were revealed to also have a genetic etiology component. Additionally, the nonsyndromic case was no longer classified as unknown. In many studies, unknown etiology accounted for 15-43% (an average of 25%) of all cases [3,13,25,27]. The percentage of our unknown cases decreased to 1.5, thanks to a detailed family anamnesis, a dismorphological examination and conduction of mutation analysis, regardless of any precondition.

Additionally, a homozygous GJB2 35delG mutation was identified in one of our sporadic cases with aminoglycosidic ototoxicity anamnesis. This case was followed for 15 years, up until the mutation analysis. His parents and unaffected sibling were revealed as heterozygous for the 35delG mutation. As Hismi et al. reported, not all individuals with homozygous 35delG have congenital severe/profound deafness [28]. It is very impressive that our case had an almost normal hearing level until the infectious high fever disease and aminoglycoside usage. This case should be taken under consideration for explanation of the phenotypic heterogeneity of the patients with homozygous 35delG mutation. It can also explain why ototoxic drugs do not always lead to deafness in children.

Of 11 cases having high fever anamnesis, 5 cases that were categorized as sporadic nonsyndromic cases also had a genetic etiology (2 cases: mitochondrial A1555G, 3 cases: GJB2 35delG mutation). For such cases, even when there are environmental causes, there may be no evidence of family amnesia or negative dysmorphology; thus the conduction of mutation analysis is very beneficial for figuring out the underlying etiology.

Out of 7 cases with mitochondrial A155G mutation, 4 were familial and 3 were sporadic, whereas out of 8 cases with the 35delG mutation, 2 were familial and 6 were sporadic cases. This finding shows that mutation analysis is also required for non-familial cases. Cochlear implants, even in advanced ages, are generally successful in cases with aminoglycosidic ototoxicity as well as in non-syndromic cases, if the patients carry a mitochondrial A1555G mutation [24,29]. Our cases promise hope in this respect.

Eight of our cases (8/64, 12,5%) had a 35delG mutation. This proportion increases to 8/39 and 20.5%, if nonsyndromic cases are also taken into account. Evirgen et al. determined the prevalence of 35delG mutation as 14.9% in nonsyndromic hearing loss cases [30]. This finding is compatible with ours. Of 8 cases with 35delG mutation, 5 were homozygous, whereas 3 were heterozygous. In addition, one of heterozygote patients with the 35delG mutation also carried a mitochondrial A1555G mutation. Among these cases, only 2 cases with homozygous mutation exhibited familial nonsyndromic hearing loss. Four of six sporadic cases had a high fever story. Of the cases with high fever history, 2 were homozygous and 1 was heterozygous for the 35delG mutation, whereas 1 was a carrier for 35delG/A1555G mutations. The case with heterozygous 35delG mutation, which did not have a high fever history or other acquired etiologic factor, may also have a mutation in GJB6 or in another gene [31]. In future studies, a sequencing analysis of the GJB2 gene and screening of the most prevalent GJB6 mutations in these patients might reveal the genetic causes. The parents of none of the patients carrying the 35delG mutation were relatives. However, parents in 2 cases were from the same village. Tekin et al. showed that the prevalence of the homozygous 35delG mutation was 2.5 times higher in children whose parents are not relatives than that of cases whose parents were first-degree relatives [15]. Moreover, this mutation was markedly more frequent in the western region of Turkey compared to the eastern region. In our cases, the 35delG mutation was more frequently observed in children whose parents were not in relatives, but who were from the same village, which is consistent with the findings of Tekin et al. [15].

The relationship between having a GJB2 mutation and benefitting from a cochlear implant is not very clear. While some studies did not report a significant relationship, some researchers reported that patients with 35delG mutation showed a significantly better prognosis [24]. As in our cases, there might be a second environmental or genetic factor aggravating the deafness phenotype in patients with 35delG mutation. In turn, the cases under the effect of a second factor might not show a better prognosis. Larger studies that analyze a variety of different mutations will be helpful.

The prevalence of deafness in Turkey (3.7/1000) is higher than developed countries, and several authors think that this situation stems from acquired/environmental factors [12,32]. However, our study showed that almost half of the cases previously diagnosed to have environmental etiology also had underlying genetic factors. In Turkey, consanguineous marriages are very common, and consanguineous marriage rates among the parents of most deafness cases are above the average. However, the parents of most of our subjects were not relatives. This situation gives rise to the idea that common alleles leading to hearing loss, such as 35delG and A1555G, might be frequent in Anatolia[33]. Only two recessive nonsyndromic 20 deaf cases had a homozygous 35delG mutation. The parents of 10 of these recessively mutated cases had a consanguineous marriage, and parents in 5 cases came from the same village. Nonsyndromic autosomal recessive cases might stem from deficiencies in other genes. Additionally, rare alleles may come together in consanguineous marriages or small size populations.

Meningitis and febrile convulsions are the most frequently observed environmental etiology. One of our previous studies was performed with 550 patients from various schools for the deaf. It was reported that out of 107 cases with environmental etiology, 30 cases had febrile convulsion, 28 cases had meningitis, and 25 cases had high fever, causing diseases such as mumps, measles and a pneumonia history [18]. Duman

et al. determined the percentages of meningitis and febrile convulsion as 28 and 5.3, respectively [19]. In this study, the hereditary and unknown etiology together accounted for 60% of the cases [19]. On the other hand, Derekoy reported febrile convulsion as the most frequent etiological factor [13]. However, these proportions may vary between countries. Walsh et al. found that the most frequent etiology among the postnatal acquired group was meningitis, and its prevalence varied between 6 and 10% [8]. Meningitis may result in bilateral or unilateral hearing loss ranging from mild to severe. Meningococus, Pneumococcus, B Hemolytic Streptoccocus and H. Influenzae are among the frequently isolated microorganisms. The prevalence of diseases that cause high fever, such as mumps and measles, decreased after the national vaccination programs. However, most of our study subjects were born and vaccinated before these programs. On the other hand, prenatal infections are not observed or are simply detected less. TORCH infections are among these prenatal incidences. Thirteen of our cases were diagnosed as having only an environmental etiology: 1 had low birth weight, 2 had prematurity, 3 had birth trauma, 1 had meningitis and 6 had a high fever history. Fifteen per cent of our cases had genetic factors in addition to acquired ones. Febrile convulsion, which is the most frequently reported etiological factor in Turkey, was detected in three of these cases. These cases were also categorized as genetic because one case was syndromic, the other case had autosomal recessive inheritance, and the last case had combined mitochondrial A1555G/GJB2 35delG mutations. Five of our cases suffered fever-causing diseases, which is another acquired factor. Three of these patients had 35delG and one had a mitochondrial mutation, while the remaining case was categorized as both a genetic and environmental case due to syndromic symptoms. Mutation analysis and the diagnosis of syndromic cases showed that almost half of the acquired cases had, in fact, a genetic etiology [8,18,19].

In conclusion, hearing loss in pediatric patients is an important medical problem since it prevents normal social and cognitive development. Infant hearing screening allows diagnosis in the first six months, and children can attend rehabilitation programs. The next step should be genetic screening and counseling services. Genetic and environmental factors play an important role in etiology. Prelingual deafness can be defined as polygenic and multifactorial, since many genes and various acquired/environmental factors are involved in the etiology. Genetic counselors should be more careful about such combined cases, since many factors interact with one another, and phenotypes and prognoses of the disease may vary from one case to another. Our results showed that dysmorphologic examinations and mutation analyses are important for the classification of etiology, and that they can provide helpful information to genetic counselors.

Acknowledgements

This study was supported by the Project of ‘Çanakkale İlindeki İşitme Engelli Olgularda Genetik Etiyoloji (Genetic Etiology of the Deaf Cases in Canakkale Region)’ Scientific Research Projects of Canakkale Onsekiz Mart University.

We thank all associations, teachers and families for their cooperation.

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