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ORIGINAL ARTICLE

Auditory Brainstem Responses in Gap Junction Beta 2 (GJB2) 35delG Mutation Carriers

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Objective: In the present study, the eight nerve and inner hair cells of the Gap Junction Beta 2 (GJB2) 35delG mutation carriers with normal hearing were evaluated by the Auditory Brainstem Response (ABR) technique.

Materials and Methods: The ABR tests were performed in 90 dB nHL, using 11 and 60/s rates and 3 polarities. Twenty-three subjects who carry GJB2 35delG mutation were compared to 25 non-carriers.

Results: Significant difference in air conduction thresholds at all frequencies was found in the left ears of both groups. In case of the right ears, significant differences were observed at all frequencies except 2 and 14 kHz. Carriers effect on ABR I, III and V wave latencies, and I-III, III-V and I-V interpeak latencies were not observed ($p<0.05$).

Conclusion: It was concluded that the GJB2 35delG mutation did not cause any damage of inner hair cells and dysfunction of the eighth nerve in carriers.

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Introduction

Congenital deafness occurs in an incidence of 1/1,000, while half of them are considered to be genetic factors [1]. A ratio of 1/3 among genetic hearing loss is accepted to be syndromic, while the rest is non-syndromic [2].

Non-syndromic hearing loss largely shows autosomal recessive inheritance. Gap Junction Beta 2 (GJB2), which encodes the Gap Junction Protein Connexin 26 (Cx26) is the cause of this type of deafness [3, 4]. Connexin 26 is found in most human tissues and various areas of the cochlea, and it plays a vital role to maintain the cochlea in its normal function [5]. Cx26 activity is shown in stria vascularis of the human cochlea, part of the spiral ligament with type I and type II fibrocytes, suprastial region, basement membrane, limbus and spiral prominence [6, 7]. Because of a very wide network of the gap junctions belonging to the sensor and support cells in the inner ear, Cx26 is found abundantly in the inner ear [8].

Mutations affecting Gap Junction Beta 2 diminish the potassium cycle and endocochlear potential. Local intoxication of the Corti organ by potassium, which is arisen from the breakdown in the potassium cycle in the cochlea, causes hair cell deaths and eventually, hearing loss [5, 8, 9]. In the cochlea and along the auditory pathway, which areas are exactly damaged by the Cx26 mutation, is not completely known [5].

Many authors have reported that 63 %-79 % of all non-syndromic hearing loss in the Mediterranean and Turkish societies was mutative [3, 10, 11]. The frequency of 35delG mutation in Turkey has been reported to be ranged from 0.8 %, to 2.7 % [12-15]. These findings agree with the other studies performed on Caucasian societies [16].

Recently, important clinical developments on early diagnosis of hearing loss, determining its degree, and pointing out the lesions have been achieved by using objective audiometric techniques [9, 17]. Pure tone air conduction hearing tests are mostly used for the evaluation of genetic hearing loss [5, 8, 9, 18-21]. Hearing tests can reveal differences in phenotypes and its etiology between individuals of the same large family, and can contribute greatly to genetic researches. Auditory brainstem evoked potentials reflect auditory nerve and brainstem afferent activity which is initiated by the inner hair cells [8]. This activity may be indicated by Auditory Brainstem Response. Morell et al. [20] have

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reported that OAEs are valuable to reveal the individuals who carry mutations causing recessive non-syndromic sensorineural hearing loss. On the other hand, even if some patients would have normal OAEs, abnormal or no ABR of the same individuals could be observed. Determination of the gene, which is responsible for hearing loss and difference between the cochlear and neural pathology, can be achieved by detailed ABR tests [17, 20].

The aim of the present study is to investigate the differences in auditory systems and influence of GJB2 35delG mutation carrier factor on the eighth nerve of healthy individuals who either carry this mutation or do not, by the implementation of ABR technique in two different speeds and three different polarities. This study was assumed that micro-level differences in auditory system of GJB2 35delG carriers and non-carriers could be determined precisely by more detailed audiological tests.

Materials and Methods

Subjects

The study was carried out with a total number of 48 subjects: 23 were heterozygous 35delG carriers (healthy carriers, 13 female and 10 male) and 25 served as a non-carrier control group (16 female, 9 male).

The relatives of fifty-nine subjects who were previously consulted about hearing loss and who were diagnosed as carriers of GJB2 35delG at the Hacettepe University, Faculty of Medicine, Department of Medical Biology were called, and 50 of them were contacted. Of the subjects who could not be contacted, three had passed away, two could not participate because of the significant health problems, and four could not be reached. Twenty-seven of the 50 subjects have accepted to be involved in the study, but three of them were found to have a mild-moderate degree of hearing loss and one had middle ear problem. Totally 23 carrier subjects were involved in the study.

Twenty-five subjects of the control group were screened for the 35delG mutation. Genomic DNA was extracted from peripheral blood according to a standard protocol. The 102 bp region covering the 35delG mutation was amplified by polymerase chain reaction (PCR) and screened by single strand conformational polymorphism (SSCP). Primer sequences were: F: 5' CAT TCG TCT TTT CCA GAG CA 3' and R: 5' TTC CAA TGC TGG TGG AGT G 3'. PCR conditions were 5 min. denaturation at 94°C followed by 35 cycles with 30 sec. denaturation at 94°C, 1 min. annealing at 58°C and 1 min. elongation at 72°C. A final extension step was applied for 5 min. at 72°C. PCR products were analyzed by polyacrylamide gel electrophoresis (PAGE) (8% non-denaturated gel, 250V, 10mA, 17 hours) and stained with silver nitrate. In each gel, sequence-tested 35delG homozygous/heterozygous and negative DNA samples were used as controls.

Subjects' age ranged between 24 and 49 (average: 35.76) in 35delG mutation carriers and 25-49 (average: 37.61) in the non-carrier control group. Subjects of both groups were planned to be included in the study who had no health problems confirmed by physical examination and had normal ear findings confirmed by otoscopic examination. Subjects who were confirmed to have an air conduction hearing threshold of better than 25 dB HL between 125-16,000 Hz bilaterally and with a middle ear pressure of ± 50 mm H2O were taken into the context of our study (Table 1).

Table 1. Demographic characteristic of study subjects

Groups	Gender			
	Male	Female	Age (Year)	Mean of Age (Year)
Carrier	10	13	24 – 49	35.76 ± 7.44
Non carrier	9	16	25 – 49	37.61 ± 8.62
Total	19	29	24 – 49	36.65 ± 7.99

Subjects were fully informed about the study and the decision of the Institutional Ethical Board of Hacettepe University.

Audiometric Evaluation

Pure tone air-bone conduction, high frequency hearing and speech tests, and threshold definitions were carried out using 'Interacoustics AC 40' (Interacoustics A/S, Assens, Denmark) audiometer equipment and inside the silent rooms (Industrial Acoustic Company Inc.) (New York, USA). Air conduction hearing tests were performed using TDH-39 standard earphones with MX41/AR casing (Interacoustics A/S, Assens, Denmark), while the bone conduction tests were done by Oticon 60273 vibrator (Oticon, Smørum, Denmark). KOSS R 80 earphones (KOSS Corporation, Wisconsin, USA) were used by the high frequency air conduction hearing tests. Pure

tone air conduction tests between 125-16,000 Hz and bone conduction tests between 500-4,000 Hz, speech reception threshold (SRT) tests, speech discrimination (SD) tests, and middle ear pressure evaluation were all carried out. Audiometer was calibrated with 4152 artificial ears using a Brüel-Kjaer sound level meter (Brüel & Kjær Sound & Vibration Measurement A/S, Nærum, Denmark).

Auditory Brainstem Response (ABR)

A "Medelec Synergy Mobile EMG/EP" (Oxford Instruments, Oxford, UK) was used for ABR testing. The golden electrodes were placed as "International 10-20 system" and their localizations were controlled by a preamplificator. Interelectrode transimpedance <4 KΩ was considered. ABRs were obtained by recording differentially between electrodes placed to the vertex (Cz), left ear A1, right ear A2 and frontal Fpz [22].

Click stimuli at an acoustic level of 90 dB nHL were applied to the right and left ears through the TDH 49P earphones (Interacoustics A/S, Assens, Denmark). ABRs were recorded at two different rates (11/s and 60/s) and three different polarities (condensation, rarefaction and alternating). Reaching to 1,500 click stimuli was used as the stopping criteria. For ABR recording band pass filter setting 30-1500 Hz was used and recording sweep time was 10 msec. Latency values of waveforms I, III and V, and interpeak latencies of waves I-III, I-V and III-V were estimated.

Statistical Analysis

The t-test was used in order to determine the groups' pure tone hearing thresholds, pure tone averages, SRT, SD, middle ear pressure and ABR. Significance at $p<0.05$ was considered, and sex was neglected.

Results

Audiometric Evaluation

Figures 1 and 2 demonstrate the air conduction pure tone hearing thresholds. The subjects of carriers and non-carriers did not suffer from hearing loss. The pure tone hearing thresholds obtained from the left and right ears were increased significantly in non carriers when compared to carriers ($p<0.05$). In the right ears non-carriers and carriers had similar pure tone thresholds at only 2,000 and 14,000 Hz.

Considering the pure tone averages (mean of 500, 1,000 and 2,000 Hz) and SRT results, significantly difference between 35delG mutation carriers and non-carriers was observed ($p<0.05$). Better pure tone averages and SRT results were obtained in non-carriers. No significant difference was found in SD between the groups.

Auditory Brainstem Response

Latencies of the peak of waves I, III and V recorded from the right ears were not significantly different between the carriers and non-carriers groups. In case of the left ears, at stimulus rate of 60/s, wave I and III latencies for ABR responses to condensation and wave III latencies to alternating polarity were significantly late in the carriers group ($p<0.05$). There was no significant difference in the wave I and III latencies at stimulus rate of 11/s. No significant group effect (carriers and non-carriers) was found on peak of wave V (Table 2).

As shown in Table 3, no significant effect of GJB2 35delG mutation on ABR interpeak latencies I-III, III-V and I-V at stimulus rates 11 and 60/s were obtained.

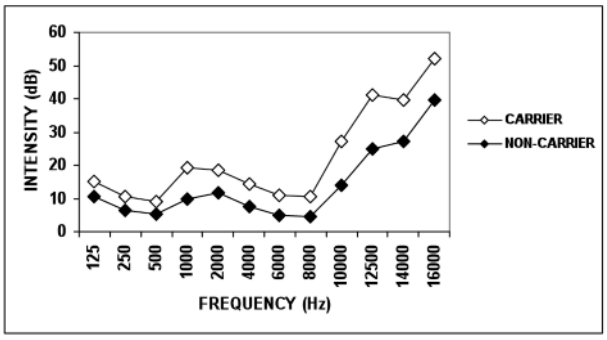

Figure 1. Pure tone hearing thresholds in the right ears of carriers (n=23) and non-carriers (n=25)

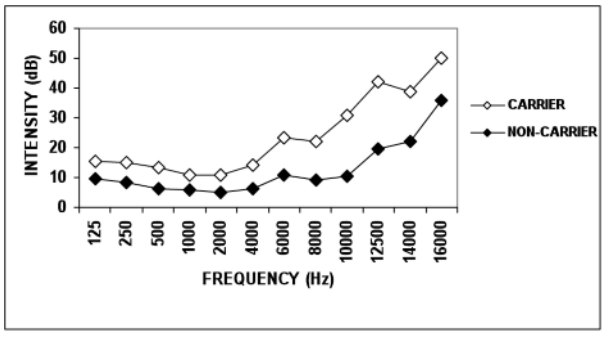

Figure 2. Pure tone hearing thresholds in the left ears of carriers (n=23) and non-carriers (n=25)

Table 2. Mean and standard deviation of I, III and V wave latencies of the groups

ABR			RIGHT EAR				LEFT EAR			
			Carrier		Non Carrier		Carrier		Non Carrier	
			Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
I	11/s	Condensation	1.64	0.15	1.61	0.17	1.68	0.16	1.65	0.21
		Rarefaction	1.65	0.10	1.64	0.17	1.64	0.17	1.64	0.20
		Alternating	1.63	0.18	1.66	0.14	1.66	0.18	1.61	0.17
	60/s	Condensation	1.78	0.18	1.74	0.22	1.80	0.22	1.65	0.20
		Rarefaction	1.77	0.16	1.77	0.25	1.74	0.23	1.68	0.14
		Alternating	1.79	0.16	1.77	0.25	1.80	0.19	1.74	0.18
III	11/s	Condensation	3.71	0.13	3.63	0.27	3.74	0.23	3.66	0.20
		Rarefaction	3.75	0.16	3.67	0.25	3.75	0.26	3.66	0.30
		Alternating	3.73	0.16	3.69	0.28	3.78	0.18	3.70	0.29
	60/s	Condensation	3.97	0.23	3.90	0.21	4.17	0.43	3.90	0.24
		Rarefaction	3.94	0.18	3.97	0.30	3.97	0.23	3.94	0.24
		Alternating	3.97	0.18	3.91	0.33	4.06	0.18	3.92	0.26
V	11/s	Condensation	5.68	0.25	5.64	0.29	5.55	0.25	5.54	0.18
		Rarefaction	5.62	0.28	5.64	0.25	5.66	0.33	5.50	0.27
		Alternating	5.59	0.26	5.68	0.28	5.64	0.18	5.57	0.28
	60/s	Condensation	5.92	0.27	5.92	0.32	6.01	0.28	5.89	0.32
		Rarefaction	5.92	0.22	5.98	0.22	5.92	0.31	5.89	0.25
		Alternating	5.98	0.25	5.97	0.31	5.97	0.25	5.90	0.32

Table 3. Mean and standard deviation of I, III and V interpeak wave latencies of the groups

ABR			RIGHT EAR				LEFT EAR			
			Carrier		Non Carrier		Carrier		Non Carrier	
			Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
I-III	11/s	Condensation	2.08	0.17	1.94	0.50	2.05	0.25	2.01	0.25
		Rarefaction	2.10	0.18	2.03	0.26	2.10	0.21	2.02	0.31
		Alternating	2.11	0.17	2.03	0.21	2.12	0.23	2.09	0.38
	60/s	Condensation	2.18	0.24	2.16	0.25	2.36	0.48	2.26	0.27
		Rarefaction	2.17	0.21	2.20	0.22	2.23	0.30	2.25	0.27
		Alternating	2.18	0.19	2.14	0.33	2.26	0.23	2.18	0.29
III-V	11/s	Condensation	1.97	0.19	2.03	0.29	1.81	0.21	1.87	0.21
		Rarefaction	1.87	0.32	1.97	0.37	1.92	0.35	1.84	0.30
		Alternating	1.86	0.19	1.99	0.27	1.86	0.17	1.87	0.41
	60/s	Condensation	1.95	0.19	2.02	0.34	1.85	0.45	1.99	0.34
		Rarefaction	1.98	0.23	2.01	0.34	1.99	0.29	1.95	0.17
		Alternating	2.01	0.23	2.05	0.34	1.91	0.22	1.98	0.37
I-V	11/s	Condensation	4.04	0.28	3.98	0.45	3.87	0.22	3.89	0.28
		Rarefaction	3.97	0.30	4.00	0.33	4.02	0.34	3.86	0.27
		Alternating	3.96	0.31	4.02	0.24	4.98	0.25	3.94	0.30
	60/s	Condensation	4.14	0.27	4.18	0.43	4.21	0.36	4.24	0.33
		Rarefaction	4.15	0.25	4.21	0.30	4.22	0.29	4.20	0.27
		Alternating	4.19	0.26	4.20	0.32	4.17	0.25	4.16	0.29

Discussion

The purpose of the present study was to investigate the differences in the auditory systems of GJB2 35delG mutation carriers. Auditory brain stem response was used to determine the effect of GJB2 35delG mutation on eight nerve and inner hair cells, by means of audiometric test batteries.

In the present study, pure tone hearing test, SRT, SD, middle ear pressure and ABR were accomplished. Pure tone hearing audiometry, SRT, SD, acoustic immitance tests, Evoked Otoacoustic Emissions and ABR has been recommended to evaluate the auditory systems of genetically hearing impaired individuals and carriers, and to determine genetically cause of hearing loss. [17, 23, 24]. The auditory test batteries are suggested to determine whether both outer and inner hair cells are completely or partially impaired [5]. Additionally, Stephens [25] has proposed the use of pure tone air conduction and high frequency hearing tests, speech audiometry, ABR, Transient Evoked Otoacoustic Emissions and vestibular evaluations to the relatives of individuals with hereditary hearing impairment [23].

Audiometric tests used to diagnose hearing loss and evaluate audiograms of homozygotes hearing impaired individuals morphologically are commonly conducted in a range of frequency between 250 and 8,000 Hz. [5, 8, 23]. According to our findings, the pure tone hearing thresholds of non-carriers were significantly higher than carriers, within the normal limits ($p<0.05$). In the right ears of non-carriers and carriers, similar pure tone thresholds at only 2,000 and 14,000 Hz. were observed.

Contrary, Engel-Yeger et al. [5] have reported that non-carriers and carriers of GJB2 35 delG had similar pure tone hearing thresholds. The difference in pure tone hearing thresholds observed in this study may be assumed to a micro-level effect of GJB2 35delG mutation on the auditory systems. High frequencies in pure tone audiometry are used for comparisons of non-carriers and carriers of GJB2 35delG mutations.[5, 25]. Decrease in high frequency pure tone thresholds of carriers may indicate that they are affected by GJB2 35delG mutation at high frequencies even if a hearing loss is not present. High frequency hearing thresholds are informative about base of the cochlea[26].

According to SRT test results, a significant difference was found between two groups for both ears ($p<0.05$). The difference in pure tone averages between the groups is expected since the method is a verification of the pure tone air conduction test. There were no significant differences in SD values between the groups, and this may show that the subjects were not considered to have retrocochlear pathologies. These findings also support our ABR results.

ABRs of the present study showed that carriers and non-carriers had the peak of waves I, III and V with similar latencies. Polarity and stimulus rate effect on ABR responses was not found. According to the results of some earlier studies, the use of click stimuli in adults with normal hearing demonstrated no polarity effects on latency on waves I, III and V [27, 28]. Conflicting results of another study has demonstrated latency differences [29]. In the present study, the waves I and III of condensation, and wave III of alternating polarity at 60/s appeared with prolonged latencies in the left ears of carriers. No significant effects of subjects groups on ABR interpeak latency differences I-III, III-V and I-V at two stimulus rates were obtained.

In their two different studies, Engel-Yeger et al. [5, 8] have tested homozygous, heterozygous, compound heterozygous carriers and non-carriers by ABR and DPOAE. The authors have reported that ABRs were absent or partial with prolonged latencies in homozygous, and ABRs of GJB2 35delG mutation carriers and non-carriers were similar. The results of the present study also confirm the same findings. It may be assumed that GJB2 35delG mutation had no effect on eight nerve and inner hair cells, and ABR tests can be a useful tool to determine whether the mutation affect these areas or not. Some studies have demonstrated that Distortion Product Otoacoustic Emissions may serve as a sensitive test which is an appropriate diagnostic tool for this mutation. Also those studies have suggested that emissions were more reliable method than ABR in order to evaluate the effect of GJB2 35delG mutation [8, 20]. Even though Distortion Product Otoacoustic Emissions are sensitive tools, ABR and high frequency hearing tests can be performed.

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