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Mutation spectrum of the connexin 26 (*GJB2*) gene in Taiwanese patients with prelingual deafness

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Purpose: To determine the mutation spectrum of the connexin 26 gene among 324 Taiwanese patients with prelingual deafness and the carrier rate of gene mutation in another 432 unrelated control subjects. **Methods:** The coding region of the connexin 26 gene was sequenced in both directions to detect mutation in all 756 samples. **Results:** Among the 756 samples tested, 21 connexin 26 variants were detected, including 7 novel ones. The 235delC mutation was the most common, accounting for 57.6% of the mutant alleles. Among patients, 48 (14.8%) had connexin 26 gene mutations. In the control group, the carrier rate of connexin 26 mutation was estimated at 2.8%. **Conclusion:** The mutation spectrum of the connexin 26 gene is wide, with more than half of the patients having only one mutation detected. Thus, further efforts are needed to look for possible existence of a second mutant allele. ***Genet Med* 2003:5(3):161–165.**

Key Words: *prelingual deafness, childhood deafness, nonsyndromic hearing loss, connexin 26 (*GJB2*) gene, connexin 30 (*GJB6*) gene*

Hearing loss affects about one in 1000 newborns.[1,2] Prelingual deafness hampers speech acquisition, normal communication, and social integration. Early identification of the condition is important for palliative treatment and special education. In developed countries, deafness in about 80% of patients has genetic causes. However, the genetic basis of deafness is highly heterogeneous, and autosomal recessive inheritance is most commonly seen.[3] Currently, about 100 loci have been linked to congenital deafness, and about 15 responsible genes have been sequenced.[4] Of these, the Gap Junction Beta 2 or the *GJB2* gene (GenBank M86849, OMIM: 121011) at chromosome location 13q12 encodes for the protein connexin 26 expressed in the cochlea and in the epidermis.[5] Connexin 26 is a gap-junction protein, which is important in cell-to-cell diffusion of small molecules. This function is necessary for recycling of potassium in the cochlea that plays an important role in sensorineural hearing function.[6,7] Currently, more than 70 mutations in this gene have been reported, most of which cause autosomal recessive nonsyndromic hearing loss, although a few cause autosomal dominant hearing loss.[4,8] Worldwide, the three most commonly reported mutations involving the connexin 26 gene are 35delG, 167delT, and 235delC.[5,8–10] Different populations have different dominant mutations.[8] Among whites, 35delG is the most common, with carrier rates ranging between 2 and 4% in Europe and in the United States.[11,12] In the Ashkenazi Jewish, 167delC is the most common mutation, with a reported carrier rate of up to 7.46%.[13,14] On the other hand, the 235delC mutation is detected in about 2% of Japanese and 1% of Korean patients with prelingual hearing loss.[15–17]

Up to 40% of patients with connexin 26 mutations have only one mutation detected.[5,8,9,11,13,15,18] In some of these patients, a 342 kb deletion, Δ(GJB6-D13S1830), has been found involving the coding region of the *GJB6* (connexin 30) gene, located at about 800 kb centromeric to the connexin 26 gene. Compound heterozygotes with one allelic connexin 26 gene mutation and one allelic Δ(GJB6-D13S1830) and homozygotes with Δ(GJB6-D13S1830) have autosomal recessive hearing loss. In Spanish patients, Δ(GJB6-D13S1830) has been reported as the second most common mutation, second to 35delG.[19] In addition to the connexin 30 gene, mitochondrial DNA (mtDNA) A1555G mutation was reported to aggravate the effect of one allelic connexin 26 gene mutation to cause prelingual hearing loss.[20]

In this article, we report the mutation spectrum of the connexin 26 gene in 342 Taiwanese patients with prelingual deafness, analyze the coding region of the connexin 30, and study the presence of Δ(GJB6-D13S1830) and mtDNA A1555G mutations in patients with only one mutation detected in the connexin 26 gene. In addition, we study the carrier rate of connexin 26 gene mutation in 432 unrelated control subjects.

MATERIALS AND METHODS

There were 324 patients with nonsyndromic prelingual hearing loss and 432 subjects with apparently normal hearing

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enrolled in this study. Among the patients, 25 were from a childhood deafness clinic, and 299 were students from a rehabilitation school. All of the students had severe to profound deafness and used sign language to communicate. The 432 hearing subjects attended our clinic to seek DNA diagnoses for reasons unrelated to deafness and skin disorders. DNA was extracted from the peripheral blood using standard methods.

With a fluorescence dye-terminator method, the coding region of the connexin 26 gene was sequenced in both directions to detect mutations in all 756 samples. To achieve this, we used a forward primer (5'-TCTTTTCCAGAGCAAACCGCC-3') and a reverse primer (5'-CCTCATCCCTCTCATGCTGTCT-3') for amplifying and sequencing a 764 base-pair (bp) fragment. Among patients in whom only one mutation was detected in the connexin 26 gene, their DNA samples were further studied for mutations in the coding region of the connexin 30 gene and mtDNA A1555G mutation by direct sequencing and for Δ(GJB6-D13S1830) mutations by gap-polymerase chain reaction (PCR). For mtDNA A1555G detection, we used a forward primer mt1229F (5'-TCAACCTCACCAC-CTCTTGCTC-3') and a reverse primer mt1727R (5'-TGGTT-TGGCTAAGGTTGTCTGG-3'). For mutation analysis of the coding region of the connexin 30 gene, we used two pairs of primers: CX30 1F (5'-CTTTGCCCACTTTTGTCTGTT-3'); CX30 1R (5'-AGGCTGCTTCAAAGATGATTCG-3'); CX30 2F (5'-CAGAAGGTTCGGATAGAGGGGT-3'); and CX30 2R (5'-CAGTTGGTATTGCCTTCTGGA-3').

PCR conditions for the connexin gene and mtDNA A1555G analyses were as follows: denaturation of genomic DNA at 95°C for 5 minutes, 35 cycles of denaturation at 94°C for 1 minute, annealing at 58°C for 1 minute and extension at 72°C for 1 minute, followed by 72°C for 10 minutes. CX30 1F and CX30 1R amplified a DNA fragment of 538 bp, and CX30 2F and CX30 2R amplified a DNA fragment of 410 bp. The mt1229F and mt1717R amplified a DNA fragment of 499 bp. All the PCR products were purified and subjected to sequencing in a semiautomated DNA sequencer (ABI PRISM 377, Perkin Elmer-Cetus, Foster City, CA). Each DNA sample was sequenced in both directions, and every electrophoregram was read by at least two of the authors.

For detection of the Δ(GJB6-D13S1830) mutation, we used primers GJB6–1R (5'-TTTAGGGCATGATTGGGGTGATT-3') and BKR-1 (5'-CACCATGCGTAGCCTTAACCATTTT-3'). Conditions for PCR consisted of denaturation of genomic DNA at 95°C for 5 minutes, 5 cycles of 96°C for 15 seconds, 68°C for 15 seconds and 72°C for 30 seconds, 25 cycles of 96°C for 15 seconds, 63°C for 15 seconds and 72°C for 30 seconds, and final extension at 72°C for 10 minutes. A positive sample was kindly supplied by Dr. Del Castillo for use as a control. Alleles with this deletion generated a 460 bp fragment.[19]

RESULTS

Twenty-one connexin 26 variants were detected in the whole series. There were four mutations (235delC, W77X, 299_300delAT, 179_191del16)[16,17,20] that were previously reported and seven novel mutations (G4D, I30 V, V43M, V63L, 184_185insT, G130A and F191L) in the patient and control groups.[7,8] 235delC was the most common, accounting for 57.6% of the mutant alleles (Table 1). In the patient group, 48 (14.8%) had mutations in the connexin 26 gene. Whereas, in the control group, nine had heterozygous 235delC, and one each had 184_185insT, 299_300delAT, and F191L. The carrier rate of the connexin 26 mutation in the control group was estimated at 2.8% (Table 1). Nineteen (5.9%) patients had two mutations (either homozygous 235delC or compound heterozygous) in the connexin 26 gene, and 29 (9%) patients had a single mutation, including 4 patients each with a reported autosomal dominant mutation (2 patients each with R75Q and R184Q, respectively) (Table 2).[21,22] Four common polymorphisms (V27I, V37I, E114G, and I203T),[17,23] one rare polymorphism (T123N), and three neutral sequence variations (H94H, T186T, V198 V) were also detected (Table 1).

Among the patients with only one mutation in the connexin 26 gene, no mutation was found in the connexin 30 coding region. Neither the Δ(GJB6-D13S1830) mutation nor mtDNA A1555G were found.

Table 1
Connexin 26 gene mutations found

Codon	Nucleotide change	Affected (648 alleles)	Control (864 alleles)	Mutation type
G4D	11G>A	7	—	Missense
V27I	79G>A	149	319	Polymorphism
I30V	88A>G	1	—	Missense
V37I	109G>A	58	100	Polymorphism
V43M	127G>A	1	—	Missense
59	176_191del16	1	—	Deletion/frameshift
62	184_185insT	—	1	Insertion/frameshift
V63L	187G>T	1	—	Missense
R75Q[a]	224A>G	2	—	Missense
W77X	230G>A	4	—	Nonsense
79	235delC	38	9	Deletion/frameshift
H94H	282C>T	1	—	Neutral sequence variation
100	299_300delAT	5	1	Deletion/frameshift
E114G	341A>G	121	275	Polymorphism
T123N	368C>A	1	14	Polymorphism
G130A	389G>C	1	—	Missense
R184Q[a]	551G>A	2	—	Missense
T186T	558G>A	—	1	Neutral sequence variation
F191L	571T>C	3	1	Missense
V198V	594G>A	1	—	Neutral sequence variation
I203T	608T>C	42	39	Polymorphism

[a]Reported as autosomal dominant inheritance.

Table 2
Distribution of mutations in the connexin 26 gene in the patient and control groups

	No. of cases	
Mutation type	Patients	Controls
235delC/235delC	11	—
235delC/W77X	3	—
235delC/299_300delAT	3	—
235delC/179_191del16	1	—
235delC/V43M	1	—
G4D/nd	7	—
W77X/nd	1	—
235delC/nd	8	9
184_185insT/nd	—	1
299_300delAT/nd	2	1
F191L/nd	3	1
I30V/nd	1	—
V43M	1	—
V63L/nd	1	—
G130A/nd	1	—
R75Q[a]	2	—
R184Q[a]	2	—
Total	48	12

nd, no mutation detected in the other allele.
[a]Reported as autosomal dominant.

DISCUSSION

Mutations in the connexin 26 gene have been detected in many populations. Although some mutations have been detected more than once, only a few have a high frequency in patients with hearing loss. In contrast with 35delG in whites and 167delT in Ashkenazi Jewish populations, the 235delC mutation is most common among Japanese and Korean patients with childhood hearing loss.[15–17,24] This study revealed that 235delC accounted for 57.6% of mutant alleles in Chinese patients with prelingual deafness, suggesting that 235delC is the most common mutation in the connexin 26 gene in oriental populations.[7,8,17,23] Two other deletion/frameshift mutations, 176_191del16 and 299_300delAT, have also been reported in Korean and Japanese patients.[15,17]

Among the 13 mutations detected in this study, 4 (235delC, 299_300delAT, 179_191del16, and 184_185insT) were frameshift mutations, 1 (W77X) was a nonsense mutation, and 8 (V43M, G4D, I30 V, V63L, G130A, F191L, R75Q, and R184Q) were missense mutations. R75Q and R184Q are reported as autosomal dominant mutations.[21,22] V43M, G4D, I30 V, V63L, G130A, and F191L are considered missense mutations based on two findings: (1) except for one F191L allele in the 864 alleles in the control group, all of the variations were detected in only the patient group, rather than in the control group; (2) across human, gorilla, orangutan, common gibbon, Rhesus monkey, cow, and mouse (accession number AF479776, AY046581, AY046582, AY046583, AY046584, AJ293886, and NM_008125, respectively), codon 43 (valine), codon 4 (glycine), codon 30 (isoleucine), codon 63 (valine), codon 130 (glycine), and codon 191 (phenylalanine) are all conserved. On the other hand, another variation T123N was considered as a polymorphism because this variant occurred only once in the patient group, but it occurred in 14 subjects in the control group (Table 1). In addition, threonine at codon 123 is not conserved, being lysine in cows and serine in dogs (accession number AJ293886 and AJ439693, respectively).

In a recent report, Wang et al.[23] studied 169 Taiwanese patients with prelingual deafness and 100 unrelated normal individuals for connexin 26 mutations. Three mutations, 235delC, 299_300delAT, and R184Q (a reported autosomal dominant mutation), were detected, and 235delC accounted for 48.3% of mutant alleles. However, no mutations were detected in the 100 control subjects. Among the 17 (10%, 17/169) patients with connexin 26 gene mutations, 12 had two mutations, and 5 had only one mutation. In our study, 13 mutations were found, and the carrier rate of the connexin 26 gene in the control group was estimated at 2.8%. Among the 49 (15.1%, 49/324) patients with mutations detected in the connexin 26 gene, 19 had two mutations, and 29 (4 with a reported autosomal dominant mutation) had one mutation. Excluding patients with autosomal dominant mutations, we found that the proportion of patients in our study (5.9%) with two connexin 26 mutations was similar to that reported by Wang et al. (7%).[23] The 2.8% carrier rate of the connexin 26 gene mutation in this study was similar to the 2% carrier rate in Japanese populations reported by Abe et al.[20]

V37I is reported as a missense mutation or a polymorphism.[10,18,20,25] In a Korean study, V37I was not detected in 147 patients and 100 control newborns.[17] In a Japanese study, V37I was detected in one patients and two control subjects.[15] However, in our study, V37I was detected in 9% of alleles of the patients and 11.6% of alleles in the hearing subjects. Wang et al.[23] had a similar finding. We believe that V37I is a polymorphism in Chinese; however, further study may be done to elucidate its nature. Two other polymorphisms in our study,[8,16,23,24] E114G and I203T (31.8% and 4.5%, respectively, by allele in the control subjects), were reported as missense mutations by Park et al.[17]

Among the five reported autosomal dominant connexin 26 mutations, W44C, G59A, D66H, R75W, and R184Q,[4,7,21,26] both R75W and R184Q were found in two unrelated patients. The available family history in the families reported here was compatible with an autosomal dominant inheritance in one family each for R75W and R184Q mutations. In the study by Wang et al.,[23] R184Q was reported as a single allelic mutation in one patient.

A large fraction (10–42%) of the patients with connexin 26 mutations have only one mutant allele.[11,15,25,27,28] Excluding the two known autosomal dominant mutations, we detected one allelic mutation in 25 (56.8%) of the patients with detect-

able connexin 26 mutations. Carriers of a single variation in the connexin 26 gene displayed evidence of reduced hair-cell function;[29] therefore, it is possible that these carriers are more likely to develop hearing loss in the presence of additional genetic or environmental factors than are noncarriers. Mutations in the DFNB1 complex (*GJB2* plus *GJB6*) and mitochondrial DNA (mtDNA) A1555G mutation have been reported to play an important role in prelingual hearing loss.[20]

Several investigations support the concept that the loss of any two of the four alleles from the connexin 26 (*GJB2*) and connexin 30 (*GJB6*) genes results in hearing loss.[30–32] Del Castillo et al.[19] identified a 342 kb deletion involving the gene encoding the connexin 30 protein. The deletion extends distally to the connexin 26 gene, which remains intact. A gap–PCR-based diagnostic method was used to detect the deletion. Two thirds of 33 unrelated probands with nonsyndromic prelingual deafness who had only one connexin 26 mutant allele were heterozygous for both connexin 30 and connexin 26 mutations. The deletion Δ(GJB6-D13S1830) was the second most frequent genetic cause (after the 35delG mutation in *GJB2*) of nonsyndromic prelingual hearing loss in the Spanish population.[19] Similar or identical deletion has been reported in Ashkenazi Jewish and French patients with hearing loss.[33,34] In our study, 56.8% of patients had only one mutation in the connexin 26 gene. Among these patients, we sequenced the coding region of the connexin 30 gene and studied the deletion Δ(GJB6-D13S1830) using a positive control DNA sample. However, all results were negative. This phenomenon may be explained by several possibilities.[1] The promoter region, exon 1, and the 4-kb intron 1 of the connexin 26 gene have not been studied. Mutations in these regions may result in abnormal transcription or splicing.[2] Deletions with different breakpoints from those of Δ(GJB6-D13S1830) may exert a similar effect on the connexin 30 or connexin 26 gene.[3] In some of our patients, the single connexin 26 mutation may have been coincidental, and defects in other genes are the real cause of hearing loss.[4] Some of the novel mutations may be autosomal dominant in nature. Further studies, including pedigree analysis and functional assays of the detected variants, are needed for clarification.

Abe et al.[20] reported mtDNA A1555G as a factor frequently associated with hearing loss in Japanese patients. A1555G mutation was detected in 8 of 23 families heterozygous either for 235delC, 299_300delAT, 179_191del 16, Y136X, or V37I. We screened for this mutation among our patients with only one mutation detected in the connexin 26 gene; nonetheless, no patient had the A1555G mutation.

Molecular analysis of the connexin 26 gene provides us with an important tool in the genetic counseling of patients with prelingual hearing loss. In cases in which connexin 26 analysis is informative, prenatal diagnosis is feasible through chorionic villus sampling or amniocentesis.[35] Although 235delC accounts for about 50% of the mutant alleles, the mutation spectrum of the connexin 26 gene is wide in Chinese patients. Direct DNA sequencing of the coding region of the connexin 26 gene may still be the most appropriate approach to identify mutations in this gene.

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