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Hearing loss associated with 35delG mutation in Connexin-26 (GJB2) gene: audiogram analysis

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Abstract
35delG is the most common mutation in the Connexin-26 gene, representing a major cause of autosomal recessive hearing loss. The aim of this study was to evaluate the relationship between the audiological phenotype and the 35delG mutation in 64 Sicilians with non-syndromic deafness. Pure-tone audiometry and a screening for 35delG mutation were performed. Audiograms were evaluated according to the classification of Liu and Xu. Thirteen homozygotes and nine heterozygotes for the investigated mutation were found. Symmetrical hearing loss was significantly ($p = 0.008$) more common in homozygous subjects than in those without the Connexin-26 mutation. Profound–severe hypoacusia was found in 92.3 per cent of 35delG homozygous, 22.3 per cent of heterozygous and 58.7 per cent of 35delG absent patients. Residual shape audiograms were more frequent in homozygotes. A molecular analysis for the 35delG mutation should be performed in cases of symmetric, severe–profound congenital hearing loss, as a genetic cause is probable in such cases.

Key words: Hearing Loss, Sensorineural; Connexins; Hearing Tests

Introduction

Prelingual hearing loss occurs in approximately 1 in 1000 live births.[1] The deficit, which is almost exclusively consequent upon defects of the inner ear, can have different causes, although it is believed to be mainly of genetic origin, with mostly autosomal recessive inheritance.[2] Mutations in the gene codifying for Connexin-26 (Cx26), GJB2, are responsible for almost half of the genetic cases of hypoacusia. More than 70 different mutations in GJB2 have been identified and, most interestingly, a single one, 35delG, accounts for up to 70 per cent of all Cx26 mutations.[3]

Initially, hearing loss related to a Cx26 deficit was reported as being only severe or profound, but recent observations have shown that a 35delG mutation may also be found in case of hearing loss of mild and moderate severity.[4–6]

Combining the clinical data reported by Cohn *et al.*[7,8] and Denoyelle *et al.*,[9–12] a consistent picture of hearing loss associated with Cx26 mutations emerges. All individuals with 35delG mutation in both alleles have a significant prelingual hearing loss, with a severity variable from mild–moderate to profound. The loss is truly non-syndromic, as other symptoms, including vestibular defects, are not apparent. Other Cx26 mutations are generally associated with more severe or profound hearing losses.[5]

A positive family history is present in only 20–30 per cent of cases, which means that the majority of cases seen in clinical practice are sporadic.[2]

Materials and methods

Subjects

We evaluated 64 Sicilian patients (29 M, 35 F), mean age 16 years (range 5–44), affected by non-syndromic hearing loss.[5] Patients were recruited between January and July 2000 and were being followed by the Audiology and Phoniatrics service since diagnosis at the Audiophoniatrics Centre of Rome. The patients were selected according to precise history and clinical criteria. The definition of non-syndromal hearing impairment was based on the absence of craniofacial abnormalities, normal paediatric and neurological evaluation, absence of cognitive defects, normal funduscopy, ECG, urine analysis and thyroid function. Furthermore, the risk of acquired hearing deficit was excluded or minimized by the use of defined indicators:

(1) history or signs of infection during pregnancy (TORCH + treponemal + HIV);
(2) birthweight <1500 g;
(3) low neonatal Apgar score (<4 at 1 min, or <6 at 5 min);
(4) need for neonatal mechanical ventilation;
(5) hyperbilirubinaemia requiring transfusion;

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TABLE I
CLASSIFICATION OF HEARING LOSS

Degree of hearing loss	Severity
Mild	Loss of 21–40 dB HL
Moderate	Loss of 41–60 dB HL
Moderately severe	Loss of 61–80 dB HL
Severe	Loss of 81–100 dB HL
Profound	Loss of more than 100 dB HL

(6) use of ototoxic medications in multiple courses;
(7) history of bacterial meningitis or other infections associated with sensorineural hearing impairment;
(8) history of trauma associated with skull fracture or loss of consciousness.

Audiological measurements

Hearing-impaired patients underwent otoscopic examination and pure-tone audiometry in a sound-proof room using a diagnostic audiometer according to ISO 389/75, ANSI S.3.13/72. As determined by tympanometry, there was no conductive component to their hearing loss. The air conduction thresholds were recorded for each ear, and the average threshold at 500, 1000, 2000 and 4000 Hz (4-PTA) of the better ear defined the severity of hearing impairment: mild (21–40 dB), moderate (41–60 dB), moderate–severe (61–80 dB), severe (81–100 dB) and profound (>100 dB), according to the Classification of Hearing Loss taken from Parving and Newton[4] (Table I).

A 15 dB difference between ears at at least two frequencies or a 10 dB difference at four frequencies defined asymmetrical hearing impairment.

Audiogram shapes were classified, according to the classification of Liu and Xu,[13] as 1) flat, 2) high-frequency (including gently sloping and sharply sloping), 3) residual and 4) specific (including U-shaped and ascending) (Table II).

TABLE II
STANDARDS FOR CLASSIFICATION OF AUDIOGRAM SHAPES (FROM LIU AND XU[13])

Type of shape	Definition
Flat	High, middle and low frequencies differ by less than 10 dBHL
High-frequency	
Sharply sloping	Either difference between total of scores at 4000, 6000 and 8000 Hz and total of scores at 500, 1000 and 2000 Hz is greater than 25 dBHL, or difference between any two octave frequencies is greater than 25 dBHL
Gently sloping	Difference between total of scores at 4000, 6000 and 8000 Hz and total of scores at 500, 1000 and 2000 Hz is 10–24 dBHL
Residual	Only residual in low frequencies
Specific	
U-shaped	Middle frequencies are worse than lower and higher frequencies by 15 dBHL or more
Ascending	Low frequencies are worse than high frequencies by 10 dBHL or more

Molecular analysis

All patients were screened for the 35delG mutation, isolating DNA from peripheral blood lymphocytes according to standard protocols.[11] Briefly, genomic DNA was extracted from peripheral blood lymphocytes of all patients using the QIAamp DNA Blood Maxi kit (QIAGEN SpA, Milan, Italy). The genomic DNA extracted was screened for the 35delG mutation by direct nucleotide sequencing, and/or by automated sequencing on an ABI prism 377 (Applied Biosystems, Warrington, UK).

If no 35delG was identified, the hearing loss was classified as not related to the 35delG mutation.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS 10.0 for Windows, SPSS Inc., Chicago, IL, USA). Data are shown as mean and standard deviation, or as median and interquartile range. Parametric (Student's *t*-test), non-parametric (Wilcoxon's test for paired data and the Mann–Whitney *U* test for non-paired data) and the χ^2 (for categorical data) tests were used to compare different values. Our criterion for statistical significance was set at p values of less than 0.05 (two-tailed).

Results and analysis

Molecular analysis results

In our sample we identified 45 (70.3 per cent) patients with a familial prelingual hearing loss, and 19 (29.7 per cent) with a sporadic prelingual hearing loss. Among patients with familial hearing loss, we found five subjects homozygous and five heterozygous for 35delG. The mutational analysis on sporadic deaf patients identified eight homozygotes and four heterozygotes for 35delG. Therefore, we found 13 (20.3 per cent) homozygous patients for the investigated mutation. Dominant, recessive and X-linked inheritance of hearing loss was investigated based on patients' family history (Table III).

Audiological results

The types of hearing loss found in the 13 35delG homozygous patients are summarized in Table IV. Residual shape was more frequent in 35delG homozygous patients and a gently sloping shape was more frequent in 35delG heterozygous subjects. χ^2 test showed the absence of a significant difference in the proportion of each audiogram shape among this study group.

Upon analysis of the distribution of degree of hearing loss, 35delG homozygous patients showed hearing thresholds significantly ($p<0.05$) poorer than those in the other two groups. In particular, profound and severe hypoacusia were found in 92.3 per cent of 35delG homozygous patients, versus 22.3 per cent and 58.7 per cent of 35delG heterozygous and 35delG absent patients (Table V). Data on our patients' hearing thresholds were not suggestive of a progressive hearing loss.

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TABLE III
DISTRIBUTION OF GENOTYPES FOR 35DELG MUTATION IN GBJ2 GENE IN FAMILIAL AND SPORADIC DEAFNESS. DOMINANT (D), RECESSIVE (R) AND X-LINKED INHERITANCE OF THE HEARING LOSS IS SHOWN

Genotype	Familiar deafness			Sporadic deafness	Total
Homozygotes for 35delG	5			8	13
	R = 5	D = 0	X = 0		
Heterozygotes for 35 delG	5			4	9
	R = 4	D = 1	X = 0		
Absence of 35delG	35			7	42
	R = 35	D = 0	X = 0		
Total	45			19	64

TABLE IV
DISTRIBUTION OF AUDIOGRAM SHAPES IN THE STUDY POPULATION

Audiogram shape	Flat	Sharply sloping	Gently sloping	Residual	Ascending	U-shaped	Total
Homozygotes for 35delG (%)	3 (23.1)	3 (23.1)	0	6 (46.1)	0	1 (7.7)	13
Heterozygotes for 35delG (%)	1 (11.1)	1 (11.1)	3 (33.3)	2 (22.2)	0	2 (22.2)	9
Absence of 35delG (%)	12 (28.6)	4 (9.5)	11 (26.2)	14 (33.3)	0	1 (2.4)	42
Total	16	8	14	22	0	4	64

TABLE V
DISTRIBUTION OF DEGREE OF HEARING LOSS IN THE STUDY POPULATION

Audiogram shape	Mild	Moderate	Moderately severe	Severe	Profound	Total
Homozygotes for 35delG (%)	0	1 (7.7)	0	3 (23.1)	9 (69.2)	13
Heterozygotes for 35delG (%)	3 (33.3)	0	4 (44.4)	0	2 (22.3)	9
Absence of 35delG (%)	1 (2.4)	6 (14.3)	7 (16.7)	12 (28.6)	16 (30.1)	42
Total	4	7	11	15	27	64

- **Mutations in the Connexin-26 gene may result in sensorineural hearing loss and are inherited with an autosomal recessive pattern**
- **This paper examines the relationship between a mutation in this connexin (35delG) and hearing loss in a cohort of patients with non-syndromic deafness**
- **Analysis showed that a symmetrical hearing loss was very common in homozygous subjects**
- **The authors conclude that a molecular analysis for 35delG mutation should be considered in patients with a severe profound inherited hearing loss**

All patients showed a symmetrical hearing loss, except for 19 without the 35delG mutation. On comparison of 35delG homozygous patients with 35delG absent subjects, the prevalence of asymmetrical hypoacusia was significantly ($p = 0.008$) higher in the group of patients without the Cx26 mutation.

Discussion

The high prevalence of 35delG among prelingual non-syndromic hearing loss patients is having a great impact on clinical practice. Audiometric shape has been considered to be a feature that may help to recognize hearing loss of genetic origin.[13,14] Some studies have questioned whether a certain type of audiogram is associated with a typical cause of hereditary hearing loss. A challenging area in non-syndromic hearing loss is the value of the audiogram in relation not only to the phenotype definition, but also to the genotype. Although several different forms of non-syndromic deafness have been discriminated on the basis of audiogram findings, the validity of these results as a guide to recognize genotypes remains unproven.

In our study the residual shape was more frequent in 35delG homozygous patients than in 35delG heterozygous patients and in those without 35delG mutation, but χ^2 test showed the absence of a significant difference in the proportion of each audiogram shape among these study groups.

As to the severity of hearing impairment, despite some recent studies, most patients homozygous for the Connexin-26 (GJB2) 35delG mutation had severe to profound hearing loss.[5–8,12–14] In fact, in our study 92.3 per cent of the homozygotes for 35delG presented a severe or profound hypoacusia, versus 58.7 per cent in the group of patients with absence of 35delG and 22.3 per cent in the heterozygotes.

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Cohn and Kelley[7,8] showed that 35delG homozygous patients can present an asymmetrical mild hypoacusia. On the contrary, none of our patients homozygous for 35delG presented an asymmetrical hypoacusia.

Conclusion

Our experience suggests that tests for prelingual non-syndromic hearing loss should be integrated in a specific audiological protocol, including a complete audiological examination of patients and their parents and, especially in those with a symmetric, severe–profound and familial hearing loss, a molecular analysis of 35delG mutation, as the first step in determining the cause of their hearing impairment. In fact, in such cases, finding this mutation is highly probable and the diagnostic investigation is relatively easy to perform. Nevertheless, it should be noted that some of the patients heterozygote for 35delG could be compound homozygotes, displaying a second different mutation in the Connexin-26 gene. This could explain the profound–severe hypoacusia found in some of the heterozygous patients. Therefore, the finding of a patient heterozygote for the 35delG mutation remains a problem in terms of genetic counselling, as it is not possible to exclude the presence of mutations other than 35delG in the Connexin-26 gene of these patients.

These results show the need for a great effort by researchers, government and pharmaceutical industries in order to give an answer to those parents who wish to know the source and recurrence risk of their children's congenital hearing impairment.

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