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Distribution and phenotype of *GJB2* mutations in 102 Sicilian patients with congenital non syndromic sensorineural hearing loss

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Abstract

Objective: To evaluate the frequency of *GJB2* mutations and their correlation with phenotype in Sicilian non-syndromic sensorineural hearing loss (NSHL) patients. *Design:* Sequencing of the coding region, basal promoter, exon 1, and donor splice site of the *GJB2* gene; screening for the presence of the two common *GJB6* deletions. *Study sample:* A cohort of 102 Sicilian NSHL patients. *Results:* Fifteen different mutations in *GJB2* and seventeen different genotypes were detected. No *GJB6* mutations were found. The hearing impairment was profound in the 64.72% of probands (mean $PTA_{0.25–4\ kHz}$ of 88.82 ± 26.52 dB HL). A total of 81.37% of patients harboured at least one c.35delG allele; c.167delT and c.-23 + 1G> A were identified in 10.78% and the 9.8% of patients respectively; c.35delG homozygotes presented more severe hearing impairment (75.59% of profound hearing loss) and a higher mean $PTA_{0.25–4\ kHz}$ (96.79 ± 21.11 dB HL) with respect to c.35delG/non-c.35delG and c.35delG/Wt patients ($P < 0.05$). *Conclusions:* This work underlines the role of c.35delG, c.167delT and c.-23 + 1G> A as the most frequent causes of NSHL in Sicily. The c.35delG frequency found is similar to those reported in other populations of the Mediterranean area. The analysis of genetic and audiologic data confirmed a variability in the phenotype associated to a single genotype.

Key Words: Sensorineural hearing loss; GJB2; Genotype-Phenotype; Sicily

Approximately 60% of congenital deafness has genetic causes in developed countries (Petit et al, 2001; Picciotti et al, 2009), with a progressive increase of this percentage due to the improvement of public health conditions and consequent reduction of hearing impairment linked to other causes such as infections (Marazita et al, 1993). Genetic sensorineural hearing loss (SNHL) is classified as non-syndromic (70%) and syndromic (30%), with an autosomal recessive mode of inheritance found in about 70–80% of patients. Lower percentages are associated with autosomal dominant (15–20%), X-linked (1%), and mitochondrial (1%) deafness.

Mutations in more than 60 genes were identified and correlated with non-syndromic sensorineural hearing loss (NSHL) (Alford 2011). Variants of *GJB2* gene, accounting for up to 50% of NSHL inherited in autosomal recessive manner, clearly play a major role in the onset of this condition (Kenneson et al, 2002). *GJB2*, expressed in the cochlea, encodes for the gap junction protein Connexin 26 (Cx26), whose function is crucial for intercellular communication, particularly in the recycling of potassium ions (K+) which occurs through a lateral and a medial transcellular pathway. In the lateral route, K^+ effluxing from outer hair cells is resorbed by Deiters' and tectal cells and flows via gap junctions through Hensen's, Claudius' and outer sulcus cells to efflux into stroma. K+ subsequently diffuses via gap junctions through type 1 fibrocytes and strial basal and intermediate cells into the intrastrial compartment. In the medial path, the K+ released by the inner hair cells travels through inner phalangeal cells, border cells, inner sulcus cells directly or by way of limbal fibrocytes via gap junctions, similar to the spiral ligament, and is finally released back into the endolymph via the interdental cells (Spicer & Schulte, 1998).

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Abbreviations

Cx	Connexin
IQR	Interquartile range
NSHL	Non-syndromic sensorineural hearing loss
PTA	Pure tone average
SNHL	Sensorineural hearing loss
Wt	Wild type

A decreased synthesis or an altered function of Cx26 may therefore reduce the efficiency of K+ circulation leading to impaired hearing sensitivity (Wangemann, 2002). Additionally, as evidenced by the c.250G> C mutation (p.V84L) studied by Beltramello et al (2005), Cx26 regulates also the permeability to Ca^{2+}-mobilizing messenger inositol triphosphate (IP3), allowing a metabolic coupling between connected cells which is necessary to generate an endocochlear potential.

Also, mutations in *GJB6*, a gene encoding for Connexin 30 (Cx30), a gap junction protein co-localized in the inner ear with Cx26, can be responsible for NSHL. Particularly, two large deletions in *GJB6* have been studied and have often been identified in trans with single *GJB2* recessive mutations, suggesting a possible recessive digenic inheritance (Del Castillo et al, 2002, 2005). *GJB6* deletions are also linked with hearing impairment in patients who carry one *GJB2* recessive mutation in trans, as a result of the abolition of an as-yet-unidentified cis-regulatory element responsible for the expression of the *GJB2* gene in the inner ear. Common et al (2005), analysing immunohistochemical data of the sweat glands of an individual compound heterozygous for c.35delG in *GJB2* and del(*GJB6*-D13D1830) in *GJB6*, observed that Cx26 expression was affected by del(*GJB6*-D13S1830). The functional effect on Cx26 protein pointed to the disruption of a putative cis-regulatory element which appears to function with cell-type specificity within the sweat gland. Rodriguez-Paris and Schrijver (2009), studying three unrelated individuals compound heterozygous for a *GJB2* mutation and del(*GJB6*-D13S1830), demonstrated that the mutated *GJB2* allele in trans with del(*GJB6*-D13S1830) was expressed, whereas the *GJB2* allele located in cis with the deletion was not expressed at all. Also del(*GJB6*-D13S1854), similar to del(*GJB6*-D13S1830), disrupts human *GJB2* expression at the transcriptional level, but, differently from del(*GJB6*-D13S1830), it is associated with low residual *GJB2* expression (Rodriguez-Paris et al, 2011).

The c.35delG mutation is by far the most common pathogenic *GJB2* allele in Caucasians, (Denoyelle et al, 1997) with a carrier frequency among European hearing individuals from 1% to 4% (Kenneson et al, 2002). A South-North gradient in c.35delG carrier frequency was observed by Gasparini et al (2000), with an average carrier frequency of 2.8% in Southern Europe, higher than Central and Northern Europe (1.3%). Particularly the Mediterranean region presents the highest c.35delG frequency with Greece representing, as suggested by Lucotte (2007), the 'geographical center' of this mutation; in Sicily, which was one of the regions of Ancient Greek colonization, Niceta et al (2007), studying 1040 newborns who underwent *GJB2* gene mutational screening, estimated a c.35delG carrier rate of 2.9%.

The main objective of this work was to study the frequency of *GJB2* mutations among Sicilian NSHL patients, trying to correlate the genotype with the severity of hearing impairment. In particular, considering the high prevalence of c.35delG found, we analysed the variability of the degree of deafness between c.35delG homozygotes and c.35delG heterozygotes. We compared also the genetic profile of our sample with those of other populations of the Mediterranean area to evidence the main differences or similarities.

Materials and Methods

Study sample

We performed a retrospective study of audiological and genetic data that we collected from 102 patients with a variable degree of hearing impairment over recent years at the Audiology Department of the University of Palermo. All the included subjects, aged between 6 and 65 years, were affected by bilateral NSHL and carried at least one *GJB2* mutation. Individuals with syndromic, unilateral or acquired hearing impairment were excluded from this study. A detailed history was taken to rule out the presence of environmental causes associated with SNHL (Joint Committee on Infant Hearing, 2007). After Ethical Committee approval, an informed consent was obtained from all participants and from parents of patients younger than 18 years old.

Clinical and audiometric evaluation

All patients underwent a physical examination of the ears through micro-otoscopy; audiometric tests and tympanograms were performed in all subjects studied. Air conduction pure-tone average thresholds at frequencies 0.5–1–2–4 kHz ($PTA_{0.5–4\ kHz}$) were calculated for each ear and were used to classify hearing loss severity. Average thresholds ranging between 21 and 40 dB HL were defined as mild hearing loss, 41–70 dB HL as moderate, 71–95 as severe hearing loss, and > 95 dB HL as profound hearing loss, as suggested by Liu et al (2005). SNHL was classified in 'symmetrical' when in presence of inter-aural threshold differences < 30 dB, and 'asymmetrical', when inter-aural threshold difference was ≥ 30 dB.

Mutation detection

After extracting DNA samples from peripheral blood, all subjects were analysed regarding the basal promoter, exon 1 and the coding region of *GJB2.*

Genomic DNA was extracted classically from peripheral blood samples with the salting-out method. Molecular analysis of *GJB2* gene was performed with PCR and direct sequencing. Exon 1 was amplified with the primers Cx1 forward (5′-TCAAAGGAACTAGGAGATCGG-3′) and Cx1 reverse (5′-CAAGGACGTGTGTTGGTCCAG-3′). Exon 2 was amplified with the primers GAP1 forward (5′-CCTATGACAAACTAAGTTGGTTC-3′) and CONN reverse (5′-GACAGCTGAGCAC GGGTTGCCTC-3′).

USB® ExoSAP-IT® PCR Product Cleanup protocol (37°C for 30 minutes and then 95°C for 5 minutes) was performed to remove leftover primers and unincorporated dNTPs (Affymetrix, Inc.).

Cleaned up PCR products were sequenced from both ends, using the same primers as used for PCR reactions, with Applied Biosystem (ABI) PRISM® BigDye® Terminator v3.1 Cycle Sequencing Kits and analysed with ABI PRISM 3100 DNA automatic sequencer.

Following PCR protocol and using primers described by Del Castillo et al (2005), the *GJB6* gene was also checked to rule out the presence of mutations del(*GJB6*-D13S1830) and del(*GJB6*-D13S1854).

Statistical analysis

Statistical analysis was performed with Matlab® computer program; χ^2 test, odds ratio (or) and/or exact test of Fisher were used,

following usual conditions of application. Group data were expressed as percentages and median (95% confidence intervals).Statistical comparisons between two groups were made using Student's paired t-test. Significance was set at 0.05.

Results

The study sample comprised 102 NSHL patients, 52 males (50.99%) and 50 females (49.01%) (Sex ratio = 1.04). With a mean age of 13.72 ± 9.75 years (median 11.5 years), the majority of participants (75.49%) were within the range of 6–17 years.

Audiologic evaluation revealed that the 95.1% (97/102) of the total population was affected by symmetric SNHL, whereas the others (4.9%) presented an asymmetric hearing loss. The majority of subjects (96.08%, 98/102) had a stable SNHL and only four persons (3.92%) presented a progressive worsening of hearing.

As shown in Table 1, all degrees of hearing loss were observed in our population. The hearing impairment was profound in 66 (64.72%), severe in 20 (19.6%), moderate in 13 (12.74%), mild in three (2.94%) patients. The mean $PTA_{0.25\text{-}4\ kHz}$ was 88.82 ± 26.52 dB HL. Specifically, it was evidenced a progressive increase in mean hearing threshold values among 0.25 kHz (73.65 ± 22.06 dB HL), 0.5 kHz (83.45 ± 23.67 dB HL), 1 kHz (93.38 ± 24.38 dB HL), 2 kHz (96.78 ± 26.6 dB HL), and 4 kHz (96.83 ± 27.79 dB HL) frequencies.

All subjects studied were positive to *GJB2* mutations; no variants in the basal promoter and exon 1 were identified. No anomalies in *GJB6* gene were found. Fifteen different mutations in *GJB2* were recognized, and seventeen different genotypes were detected in our study sample.

Of the 102 patients of our cohort, 83 (81.37%) harboured at least one c.35delG mutation, 11(10.78%) a c.167delT, and 10 (9.8%) a c.-23 + 1G> A; c.35delG, c.167delT, and c.-23 + 1G> A represented respectively the 76.3% (132/173), 6.93% (12/173) and 5.78% (10/173) of all mutated *GJB2* alleles. Each remaining mutation observed involved less than 4% of patients.

We classified c.35delG carriers in three subgroups: the first (G_1), composed by 49 patients who were homozygous for the c.35delG mutation; the second (G_2), and the third (G_3) respectively constituted by 15 subjects carrying a c.35delG mutation associated with other *GJB2* mutations in trans (c.35delG/ non-c.35delG), and 19 patients with a c.35delG/Wt genotype (Table 1).

The study of the audiologic profile of G_1 group revealed a mean $PTA_{0.25\text{-}4\ kHz}$ of 96.79 ± 21.11 dB HL (median = 100 dB HL), with the 53.06% (26/49) of audiograms characterized by a 'flat loss' while the 46.94% (23/49) consisted in a 'sky slope' curve; thirty-nine subjects were affected by profound, eight by severe, and two by moderate SNHL (Figure 1).

Concerning G_2 patients, four different genotypes were recognized (c.35delG/c.-23 + 1G> A, c.35delG/c.269T> C, c.35delG/c.167delT, c.35delG /c.535 G to C) with a mean $PTA_{0.25\text{-}4\ kHz}$ of 73.8 ± 27.41 dB HL. A total of 53.33% of subjects (8/15) presented a flat, 26.66% (4/15) an 'U shaped', and 20.01% (3/15) a sloping audiogram; ten patients had a severe to profound hearing impairment, whereas five individuals were affected by mild to moderate SNHL.

From the analysis of the audiologic characteristics of G_3 group it resulted in a mean $PTA_{0.25\text{-}4\ kHz}$ of 87.78 ± 26.9 dB HL; the majority of audiograms were represented by sloping curves (47.38%, 9/19), followed by flat and 'U shaped' audiograms in 36.84% (7/19) and 15.78% (3/19) of cases respectively; eleven probands were affected by profound, four by severe, three by moderate and only one by mild SNHL.

With a prevalence of 79.59% (39/49), 40% (6/15), and 57.89% (11/19) of profound hearing loss among G_1, G_2, and G_3 groups, the study of the audiologic phenotype revealed a statistical significant difference between these groups (P = 0.012). Distribution of hearing thresholds (for 0.25–0.5–1–2–4 kHz) relative to G_1, G_2, and G_3 groups is reported in Figure 2. It is well shown that the highest mean $PTA_{0.25\text{-}4\ kHz}$ values were associated with c.35delG homozygous genotype, with a statistical significant difference with respect

Table 1. Frequency, hearing loss degree, and hearing threshold of all genotypes identified in the cohort.

		Hearing loss degree				*Hearing threshold (dB HL)*		
Genotypes	*N(%)*	*Mild*	*Moderate*	*Severe*	*Profound*	*Mean*	*Standard deviation*	*Median*
35delG/35delG (G_1)	49(48.03)	0	2	8	39	96.79	± 21.11	100
35delG/non-35delG (G_2)	15(14.7)	1	4	4	6	73.8	± 27.41	80
35delG/Wt (G_3)	19(18.62)	1	3	4	11	87.78	± 26.9	90
35delG / -23 + 1G>A	6(5.88)	0	1	4	1	65.33	± 24.06	70
35delG/167delT	5(4.9)	0	1	1	3	86.6	± 17.95	90
35delG/269T>C(L90P)	3(2.94)	1	1	0	1	43.66	± 19.86	45
167delT/Wt	3(2.94)	1	0	0	2	82.33	± 39.77	95
457G>A(V153I) /Wt	3(2.94)	0	1	1	1	80.33	± 23.71	80
167delT/139G>T(E47X)	2(1.96)	0	0	0	2	105.5	± 16.4	110
-23 + 1G>A/Wt	2(1.96)	0	1	1	0	53	± 19.6	47.5
101T>C(M34T)/Wt	2(1.96)	0	1	0	1	79	± 29.51	77.5
313del14/-23 + 1G>A	2(1.96)	0	0	1	1	87	± 21.49	80
167delT/167delT	1(0.98)	0	0	0	1	107	/	/
35delG/535G>C(D179H)	1(0.98)	0	0	0	1	103	/	/
310del14/551G>C(R184P)	1(0.98)	0	0	0	1	104	/	/
101T>C(M34T)/269T>C(L90P)	1(0.98)	0	1	0	0	48	/	/
380G>A(R127H)/Wt	1(0.98)	0	0	0	1	106	/	/
301del3/Wt	1(0.98)	0	1	0	0	46	/	/
Total*	102	3	13	20	66	88.82	± 26.52	90

*With the exclusion of c.35delG/non-c.35delG genotype.

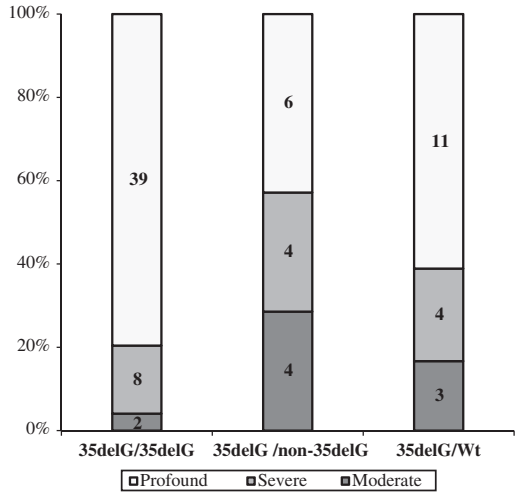

Figure 1. Relative frequencies of the degree of hearing loss in c.35delG/c.35delG, c.35delG/non-c.35delG, and c.35delG/Wt genotypes. Mild SNHLs were not reported.

to G_2 group for all frequencies studied ($P < 0.01$) and to G_3 limited to 1–2–4 kHz frequencies ($P \leq 0.05$).

The study of a box plot (Figure 2) for each group revealed a lower variability in the degree of SNHL among G_1 patients (mean $IQR_{0.25\text{-}4\ kHz} = 26$ dB HL) than among G_2 (mean $IQR_{0.25\text{-}4\ kHz} = 41.5$ dB HL) and G_3 subjects (mean $IQR_{0.25\text{-}4\ kHz} = 40$ dB HL). With a prevalence of 79.59% (39/49), 40% (6/15), and 57.89% (11/19) of hearing loss ≥ 90 dB HL for 2–4 kHz frequencies, it resulted a statistical significant difference between G_1, G_2, and G_3 ($P = 0.012$); additionally, concerning 0.25 and 0.5 kHz frequencies, G_2 is associated with a higher variability in the distribution of data and a greater percentage (46.66%, 7/15) of patients affected by mild to moderate hearing loss than G_1 ($P = 0.032$).

The c.167delT mutation was found in 11 probands (10.78%), associated with four different genotypes: c.167delT homozygous, c.167delT/c.35delG, c.167delT/c.139G> T, c.167delT/Wt. From the study of the audiologic data of all c.167delT carriers it resulted in a mean $PTA_{0.25\text{-}4\ kHz}$ of 90.18 ± 26.49 dB HL and a profound SNHL in the majority of cases (72.72%, 8/11). Particularly, the five subjects with a c.167delT/c.35delG genotype presented a variable degree of hearing loss (one moderate, one severe, and three profound) and a mean $PTA_{0.25\text{-}4\ kHz}$ of 86.6 ± 17.76 dB HL, lower than non c.35delG /c.35delG carriers but without any significant difference ($P = 0.067$).

The c.-23 + 1G> A mutation was identified in 10 patients (9.8%) and distributed in three genotypes (c.-23 + 1G> A /c.35delG, c.-23 + 1G> A /c. 313del14, c.-23 + 1G> A /Wt; two probands were affected by profound, four by severe, three by moderate, and one by mild SNHL, with a mean $PTA_{0.25\text{-}4\ kHz}$ of 67.2 ± 24.8 dB HL, lower than that of c.167delT carriers ($P = 0.006$).

Discussion

Among more than 90 different *GJB2* mutations c.35delG accounts for up to 75% of mutated alleles in populations with European ancestry (Gasparini, 2000; Oguchi et al, 2005). In the Mediterranean area, with a great diffusion in the general population (carrier frequency of ~1/30), c.35delG represents the most common pathological *GJB2* allele associated with NSHL (Lucotte et al, 2005). We

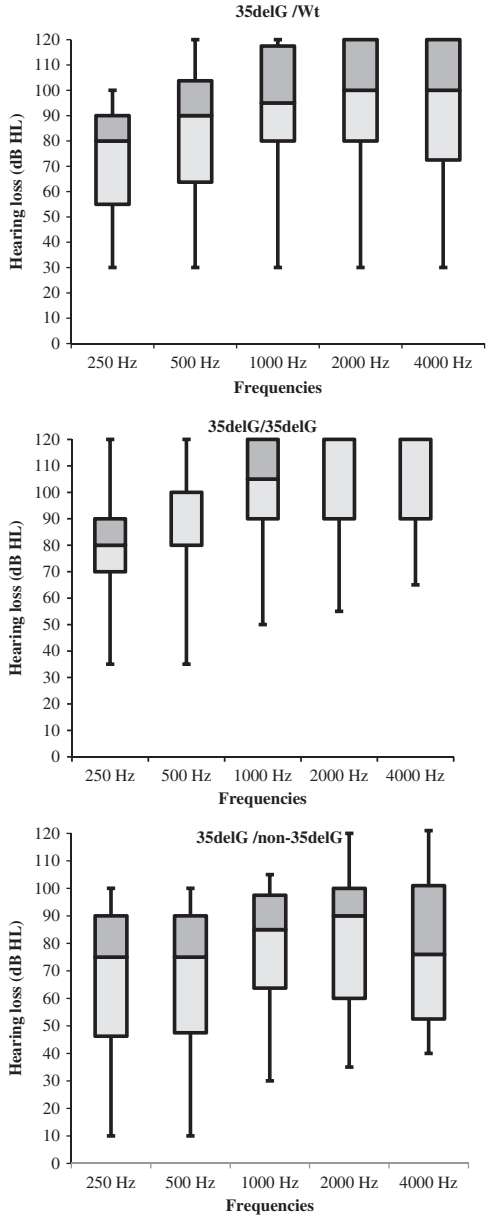

Figure 2. Box plot. Distributions of PTA 0.25–4 kHz of patients with c.35delG/c.35delG, c.35delG/non-c.35delG, and c.35delG/Wt genotypes.

evidenced in fact a c.35delG frequency of 76.3% among the total mutated *GJB2* alleles (132/173), in line with data relative to Tunisia (85.4%), Spain (82%) Algeria (76%), Turkey (76%), France (69%), Portugal (85%), Morocco (90.8%), and Greece (95.2%) (Rabionet et al, 2000; Pampanos et al, 2002; Kalay et al, 2005; Marlin et al, 2005; Abidi et al, 2008; Ammar-Khodja et al, 2009; Chora et al 2010; Rihai et al, 2013).

It is important to underline that *GJB6* mutations, which were not found in our cohort, represent only a small percentage of DFNB1 mutations in our country; in fact, as demonstrated by many studies published previously, *GJB6* mutations were not or sporadically recognized in Italian NSHL patients, with an evident difference between Northern and Southern Italy (Del Castillo et al, 2003; Gualandi et al, 2004). Chinetti et al (2011), for example, analysing the genotype of

129 hearing-impaired infants from the Campania region, did not evidence any *GJB6* mutations; Primignani et al (2009) and Berto et al (2009) instead found *GJB6* mutations only in three (0.4%) and five patients (1.3%) of two cohorts from Northern Italy composed respectively by 734 and 385 patients. On the contrary, in other countries of Western Europe such as Portugal and France, higher percentages of these mutations were observed (7.4% and 8.2% respectively) and also in Spain, where the *GJB6* mutations represent the second most frequent cause of prelingual deafness (Del Castillo et al, 2003); Del Castillo et al (2002) described previously this peculiar geographical distribution of *GJB6* mutations, especially of del(*GJB6*-D13S1830), for which they suggested a 'common founder' to justify the greater prevalence in certain populations.

In the 48.04% (49/102) of c.35delG homozygotes in our cohort, 95.92% (47/49) had a severe to profound degree of hearing loss and a median $PTA_{0.5\text{-}2\ kHz}$ of 100 dB HL, similar to data reported by Hismi (90.9% of severe to profound HL and a median $PTA_{0.5\text{-}2\ kHz}$ of 104 dB HL) and Cryns (91.56% of severe to profound HL and a median $PTA_{0.5\text{-}2\ kHz}$ of 102 dB HL) (Cryns et al, 2004; Hismi et al, 2006). Additionally, also from the comparison of our results with those of Iliadou (100% of severe to profound HL and a mean $PTA_{0.5\text{-}2\ kHz}$ of 99.78 dB HL) and Santos (100% of severe to profound HL and a mean $PTA_{0.5\text{-}2\ kHz}$ of 101.5 dB HL), no significant difference was evidenced with respect to our data (Iliadou et al, 2004; Santos et al, 2005). However, even if c.35delG/c.35delG was the most represented genotype among the studies cited, a certain variability in its prevalence emerged. Hismi et al (2006) and Cryns et al (2004), for example, found higher frequencies of this genotype (69.8% and 60% respectively), differently from our work (48.03%, 49/102) and from Iliadou et al (2004) and Santos et al (2005) (25.2% and 47.36% respectively). This discrepancy could be explained considering that the first two authors cited included in their works only subjects carrying biallelic *GJB2* mutations. In fact, by excluding patients carrying only one *GJB2* mutation, the c.35delG/c.35delG percentage found in our cohort increases to 69.01% (49/71). Furthermore, it is clear that there is a correlation between this genotype and an audiological phenotype characterized by severe to profound hearing loss, even if, cases of mild to moderate SNHL were previously reported (Wilcox et al, 2000; Snoeckx et al, 2005; Hismi et al, 2006) and were also observed in our sample (2/49 of c.35delG/c.35delG carriers). The presence of different degree of hearing impairment in patients carrying the same *GJB2* mutations is not clearly understood, but many authors underlined the role of environmental factors or modifier genes as potential causes of this variability (Hismi et al, 2006; Hilgert et al, 2009; Picciotti et al, 2009).

We compared the audiological phenotypes of patients homozygous for c.35delG (G_1), c.35delG/non-c.35delG (G_2), and c.35delG/Wt carriers (G_3). From this analysis a more severe hearing impairment in G_1 subgroup ($P = 0.012$) emerged, with a statistical significant difference with respect to G_2 group for all frequencies studied ($P < 0.01$) and to G_3 limited to 1–2–4 kHz frequencies ($P < 0.05$); G_2 instead was characterized by a higher percentage (46.66%, 7/15) of patients affected by mild to moderate hearing loss with respect to G_1 patients ($P = 0.032$). The study of distribution of $PTA_{0.25\text{-}4\ kHz}$ hearing thresholds showed in Figure 2 clearly reveals a close hearing loss spectrum for G_1 patients, with an accumulation of values around 100–120 dB HL, especially for high frequencies.

Concerning c.167delT, the second most frequent mutation recognized in our cohort (10.78%, 11/102), it was observed associated with c.35delG in the 45.45% (5/11) of cases and was characterized by a moderate to profound hearing loss (mean $PTA_{0.25\text{-}4\ kHz}$ of 90.18 ± 26.49 dB HL); a statistically significant difference emerged between the degree of hearing impairment of c.167delT/c.35delG and c.35delG/ c.-23 + 1G> A compound heterozygotes ($P = 0.03$). The c.167delT/c.35delG genotype was carried by 4.9% (5/102) of our patients, with a median $PTA_{0.5\text{-}2\ kHz} = 90$ dB HL; our data are comparable to Cryns et al (2005), Marlin et al (2005), Snoeckx et al (2005), and Hismi et al (2006) who reported lower frequencies (< 4%) of c.167delT/c.35delG carriers but with similar degree of hearing impairment (mainly severe to profound).

We found the c.-23 + 1G> A mutation in the 9.8% (10/102) of our cohort with a mild to profound spectrum of hearing loss; lower percentages were evidenced by Cryns et al (2.89%), Snoeckx et al (< 1%), and Berto et al (1.7%) studies (Cryns et al 2005; Snoeckx et al, 2005; Berto et al, 2009). These studies identified this mutation mainly in the heterozygous state and associated with c.35delG. From the comparison of the audiological profile of our c.-23 + 1G> A /c.35delG patients (median $PTA_{0.5\text{-}4\ kHz} = 70$ dB HL) with literature data, the median hearing threshold seems to be superimposable; in fact Cryns et al (2005) and Snoeckx et al (2005) evidenced respectively median values of 62 dB HL and 64 dB HL among 7 and 16 patients with a c.35delG/ c.-23 + 1G> A genotype. On the contrary Santos et al (2005) described two cases of profound SNHL associated with c.35delG/ c.-23 + 1G> A (mean $PTA_{0.5\text{-}2\ kHz} = 97.9$ dB HL); also in our cohort one patient was identified with profound SNHL. All these data seems to be contradictory, reflecting a variability in the phenotype associated to a single genotype and are not explainable on the basis of functional studies; in fact, D'andrea et al (2002) and Shanin et al (2002) demonstrated, for c.35delG and c.-23 + 1G> A, do not yield detectable Cx26 protein and mRNA respectively. This is inconsistent with the observation made by Snoeckx et al (2005) and supported by our data, that c.35delG/ c.-23 + 1G> A compound heterozygotes had significantly less severe hearing impairment compared to c.35delG homozygotes ($P < 0.0001$).

In conclusion, the present study, analysing the genetic profile of NSHL Sicilian patients, highlights the role of certain *GJB2* mutations (c.35delG, c.167delT, c.-23 + 1G> A) as the most frequent causes of congenital deafness in Sicily. Also from the study of our data, it emerged a real difficulty to identify precise genotype-phenotype correlations, even in cases of certain genotypes (as c.35delG homozygotes) which are mainly associated to a determinate degree of hearing impairment. Future research is necessary to provide additional data that could improve the knowledge about the effects of modifier genes and environmental factors in the variability of audiologic phenotype.

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