Biol Res 46: 239-242, 2013

Prevalence of the 35delG mutation in the GJB2 gene in two samples of non-syndromic deaf subjects from Chile

Lucía Cifuentes[1], Margarita Arancibia[2], Mariela Torrente[4], Mónica Acuña[1], Corina Farfán[3], Carolina Ríos[1]

[1] Laboratorio de Epidemiología Genética, Programa de Genética Humana, ICBM. Facultad de Medicina, Universidad de Chile.
[2] Servicio de Otorrinolaringología, Hospital San Juan de Dios, Santiago, Chile.
[3] Escuela de Tecnología Médica, Facultad de Medicina, Universidad de Chile.
[4] Departamento de Otorrinolaringología, Universidad de Chile.

ABSTRACT

Hearing loss is the most common inherited sensorial deficiency in humans; about 1 in 1000 children suffer from severe or profound hearing loss at birth. Mutations in the GJB2 gene are the most common cause of prelingual, non-syndromic autosomal recessive deafness in many populations; the c.35delG mutation is the most common in Caucasian populations. The frequency of the c.35delG mutation was estimated in two samples of deaf patients from Santiago, Chile. Unrelated non-syndromic sensorioneural deaf patients were examined: Group 1 consisted of 47 unrelated individuals with neurosensory deafness referred to the Chilean Cochlear Implant Program; Group 2 included 66 school children with prelingual deafness attending special education institutions for deaf people. Individuals with profound to moderate isolated neurosensory hearing loss with unknown etiology were included. The presence of the c.35delG mutation was evaluated by the allele-specific polymerase chain reaction method (PCR), and in some cases it was confirmed by direct DNA sequencing of the coding region of the GJB2 gene. Deaf relatives were present in 20.3% of the cases. We found 19.5% (22/113) patients with the c.35delG mutation, 6 of them homozygous; these rates are similar to frequencies found in other Latin American countries.

Key words: Genetic deafness, Hereditary hearing loss, 35delG mutation frequency, GJB2 mutations, Chilean population.

INTRODUCTION

Hearing loss is the most common inherited sensory deficiency in humans; approximately 1 in 1000 children suffer from severe or profound bilateral permanent hearing loss at birth or during early childhood (Morton, 1991). A congenital hypoacusis rate of 2.8 per 1000 newborns has been reported in Chile (Nazar et al., 2009). In developed countries, around 60% of the cases of childhood deafness have a genetic origin and 70% of them are non-syndromic forms (Petit et al., 2001; Marazita et al., 1993). The most common pattern of inheritance among hereditary non-syndromic deafness is autosomal recessive, about 80% (Zelante et al., 1997). To date, numerous loci (about 60) have been involved in this kind of deafness (http://hereditaryhearingloss.org 2013); however, the mutations in the GJB2 gene (encoding the gap-junction protein connexin-26 in the DFNB1 locus in 13q12) are the major cause of prelingual, non-syndromic autosomal recessive deafness in many populations (Denoyellle et al., 1999; Kenneson et al., 2002; Álvarez et al., 2005; Gravina et al., 2,010). About one hundred GJB2 mutations have been associated with autosomal recessive non-syndromic deafness (http://davinci.crg.es/deafness 2013). Different mutations are found among populations from different ethnic backgrounds. One of these, c.35delG, consists of a deletion of one guanine in a sequence of six guanines, leading to a frame shift and premature stop of the synthesis of connexin-26; this mutation has a high prevalence in Caucasian populations (Petit et al., 2001; Denoyelle et al., 1999), accounting for 70% of all pathogenic GJB2 alleles (Denoyelle et al., 1999: Cryns et al., 2004). Analyses of the GJB2 gene in patients with autosomal recessive inherited deafness, especially due to the c.35delG mutation, have shown that 10% to 50% of them presented only one mutant allele (Kenneson et al., 2002). Studies of these heterozygous patients for GJB2 mutations have found other mutations in the GJB6 gene, which encodes connexin-30 (also located at the DFNB1 locus, 35 kb from the GJB2 gene); in these cases there is a digenic origin of deafness (Del Castillo et al., 2005, Cordeiro-Silva et al., 2011).

Among the Italian and Spanish populations a carrier frequency of the c.35delG mutation of 3.2% has been found (Estivill et al., 1998); however, this mutation has not been found in Asian populations (Kudo et al., 2000), where the c.235delC is the most prevalent (Abe et al., 2000; Park et al., 1999). No individuals with the c.35delG mutation were found among 365 students with profound sensorineural hearing loss in Ghana, Africa (Brobby et al., 1998).

Some studies have been conducted in Latin American populations: e.g. two studies in Brazil reported c.35delG frequencies of 12.4% and 11.8%, respectively among non-syndromic deaf patients (Batissoco et al., 2009; Cordeiro-Silva et al., 2011), while the frequency of c.35delG carriers in the Brazilian general population was 0.94% (Nivolini et al., 2010). In Venezuela, the c.35delG was found in 27.5% of deaf children and the carrier frequency was 4% (Utrera et al., 2007). Studies in deaf children from Argentina reported that c.35delG, the most frequent mutation, was present in 26.5% and 24.0 % of the cases (Gravina et al., 2010; Dalamón et al., 2005).

There are two local preliminary publications related to GJB2 mutations in the Chilean population: Arancibia et al. (2012) found the c.35delG mutation in 11.25% of deaf school children and López et al. (2012) found two patients heterozygous for the c.35delG mutation among 8 patients

Corresponding Author: Lucía Cifuentes. Programa de Genética Humana, ICBM. Facultad de Medicina. Universidad de Chile. PO Box: Casilla 70061- Santiago 7-Chile. Telephone: 56-2-29786011. FAX: (56-2) 27373158. Email: lcifuent@med.uchile.cl

Received: March 1, 2013. In Revised form: May 13, 2013 . Accepted: May 17, 2013.

with congenital nonsyndromic sensorineural deafness. The current Chilean mixed population is of biracial origin, it was generated by the admixture between aboriginal populations (Amerindians of Asian origin) and Spanish conquerors of European origin that arrived in the country in the latter part of the sixteenth century (Valenzuela et al., 1987; Cifuentes et al., 2004). The aim of this study was to estimate the frequency of c.35delG in a sample of deaf patients from Santiago, Chile, and compare the findings to other populations with similar ethnic background.

MATERIALS AND METHOD

Patients

We examined two groups of unrelated non-syndromic sensorineural deaf patients from Chile: Group 1 consisted of 47 unrelated individuals with neurosensory deafness referred to the Chilean Cochlear Implant Program at the Otolaryngology Service of the Barros Luco Hospital in Santiago, arriving from all over the country with ages from 2 to 39 years. Group 2 included 66 school children with prelingual hearing loss attending three special education institutions for deaf people in Santiago, the capital city of Chile (Jorge Otte School, Anne Sullivan School, and San Francisco School); the children ages ranged from 6 to 22 years at the moment of the study (Arancibia et al., 2012). The inclusion criterion for the study was profound to moderate isolated neurosensory hearing loss with unknown etiology; thus individuals with syndromic deafness or secondary acquired deafness (caused by trauma, ototoxic drugs, prematurity or infectious diseases) were excluded. Clinical information was obtained from the individuals' medical and school records, as well as a questionnaire answered by their parents in case of children. We excluded those cases with incomplete clinical records. The protocol followed the Declaration of Helsinki guidelines and was approved by the Ethics Board of the Universidad de Chile School of Medicine; an appropriate informed consent was obtained from all participants or their parents in the case of minors.

Molecular genetic analysis

Genomic DNA was extracted by a salting out protocol (Miller et al., 1998) from peripheral blood leukocytes in Group 1 and from buccal cells collected on buccal swabs in Group 2.

After DNA isolation, all samples were evaluated for the presence of the c.35delG mutation using the allele-specific polymerase chain reaction (PCR). Briefly, two PCR amplifications of genomic DNA of each sample were performed, one to identify the normal allele and the other to identify the c.35delG mutation. Specific primers for the normal or the c.35delG mutation and the common primer were used (Scott et al., 1998).

All cases from group 2 harboring the c.35delG mutation and a random sample of 15 cases with wild genotype were further analyzed by direct sequencing of exon 2 of the *GJB2* gene. Exon 2 of the connexin 26 gene was sequenced after DNA amplification with a set of external primers (5′ TGCTTACCCAGACTCAGAGAA 3′ – 5′ CGACTGAGCCTTGACAGCTGA 3′) and a set of internal primers (5′ AGTGGCCATGCACGTGGCCTA 3′ – 5′ TGATCTCCTCGATGTCCTTAA 3′) according to Wu et al., 2003. Chromatograms obtained after sequencing were analyzed according the information available in the NCBI Gene Bank (Access number: M86849)

Statistical analysis

The 35delG frequencies were compared by a Chi square test. The statistical significance level was 0.05.

RESULTS

A total of 113 patients (90 sporadic and 23 familial cases) with idiopathic non-syndromic sensorineural hearing loss were genotyped for the c.35delG mutation at the GJB2 gene. Among these, 46 were males and 67 were females. Profound deafness was more frequent (84 patients) than severe (16 patients) and moderate cases (3 patients). Table I presents the results obtained in the c.35delG genotyping, according to familial records of deafness. We found 22 patients (19.5%) who carried the c.35delG mutation (95% CI: 12.2 - 26.8%); six were homozygous for the mutation; accordingly, we could establish the etiology of deafness in these 6 cases. The c.35delG mutation was more frequent in familial cases of deafness (65%) than sporadic cases (7.8%), p = 0.00025. The frequency of the c.35delG pathogenic allele in the sample was 12.4% (95% CI: 10.2% - 14.6%). In addition we found the non-pathogenic variant V27I in 7 deaf children.

TABLE I

Characteristics of non-syndromic sensorineural deaf patients from Chile

	Sporadic cases		Familial cases		Total
	n	%	**n**	%	
c.35delG/c.35delG	2	2.2	4	17.4	6
c.35delG/N	5	5.6	11	47.8	16
N/N	83	92.2	8	34.8	91
Total	90	100.0	23	100.0	113

N= absence of c.35delG

DISCUSSION

As in many other countries, our study demonstrated that the c.35delG mutation is a frequent cause of hearing impairment in Chile. Also, as is expected in a hereditary disease, this mutation was more frequent in familial than sporadic cases of deafness.

We could explain the etiology of deafness in only 6 cases that were homozygotes for the 35del G mutation. The presence of a single pathogenic allele does not explain the cause of deafness, therefore other mutations in connexin 26 or in other related genes may be associated with the deafness in the 16 patients that carried only one 35delG allele.

This situation is not uncommon; in fact, several studies in patients with autosomal recessive deafness have shown that about 10% to 50% of them presented only one mutated allele

in the GJB2 gene (Kenneson et al., 2002). The finding that there is an important number of non-syndromic sensorineural deaf persons carrying a single GJB2 mutation led to a search for other mutations in or near GJB2. Del Castillo (2002, 2005) identified two large deletions in the connexin 30 gene: GJB6-D13S1830 and GJB6-D13S1854; these deletions are usually found accompanying the GJB2 coding mutation, causing the deafness.

The deletion del(GJB6-D13S1830) was the accompanying mutation in about 50% of Spanish patients with only one GJB2 coding mutation (Del Castillo, 2005). Latin American studies have found the D13S1830 deletion in 11% to 37% of patients with non-syndromic sensorineural deafness that carried only one copy of the c.35delG mutation (Batissoco et al., 2009; Cordeiro-Silva et al., 2011; Dalamón et al., 2005; Gravina et al., 2007; Utrera et al., 2007). López et al. (2012) did not identify GJB6 deletions in the 8 patients they studied. The D13S1830 deletion accounted for the deafness in 15.9% of the patients with only one GJB2 mutation in a study performed by Pandya (2003) in the USA. Even if the whole coding region of the GJB2 gene and the known mutations at the GJB6 genes were studied, there still would be cases with a monoallelic pathogenic mutation that is not enough to explain the deafness.

Our patients with only one 35delG mutation could be compound heterozygous with another mutation in the GJB2 gene, either in the coding region or outside of it in the promoter or splicing recognition site. Moreover, some of these patients with only one 35delG mutation could have another mutation at the GJB6 gene (digenic inheritance).

The genetic heterogeneity of non-syndromic hearing loss makes its molecular diagnosis difficult, given the number of mutations that have been described in deafness-related genes. Additionally, we have to consider the difficulties in distinguishing between genetic and non-genetic deafness in families presenting a single deaf subject (90 cases in our sample -Table I), notwithstanding the efforts to find possible environmental causes of deafness in their clinical records.

In this study, as in many others, we found no explanation for the deafness in several heterozygous patients. Some studies regarding mutations in the GJB2 gene have been performed in other mixed Latin-American countries with a Caucasian ethnic component (comparable to the Chilean population). These studies had the same inclusion and exclusion criteria (syndromic hearing loss, probable environmental cause of deafness, transmission deafness) to select the patients to be analyzed. Table II compares their results with our study: we see that the frequency of the c.35delG mutation (in biallelic or monoallelic situations) among non-syndromic deaf patients from Santiago, Chile is in line with the frequencies found in the majority of other Latin American countries.

Comparing the c.35delG frequency among these different studies, we find that three of them are different from the rest ($p = 0.043$): a higher frequency in Argentina (Dalamón et al., 2010) and a lower frequency in Mexico (Arenas-Sordo et al., 2012) and Brazil (Cordeiro-Silva et al., 2011); the remaining studies, including our study in Chile, found similar results. The proportion of homozygotes for the c.35delG mutation among those carrying this allele was similar in all the studies cited in Table II ($p = 0.23$).

We can conclude that the c.35delG mutation plays an important role in the etiology of hearing loss in Chile, as in other countries with similar genetic backgrounds; however, there is need of further research to find other pathogenic alleles involved in genetic hearing loss in our country.

AKNOWLEDGEMENTS

We thank the Sociedad Chilena de Otorrinolaringología, Medicina y Cirugía de Cabeza y Cuello of Chile for financial support. We also thank all the persons who consented to participate in this study and Dr. Daisy Pezoa for the ascertainment of patients from the Chilean Cochlear Implant Program.

TABLE II

Studies performed to detect the c.35delG mutation in non-syndromic sensorineural deaf patients from different countries from Latin America

Country	n	c.35delG/c.35delG	c.35delG/N	Reference
		%	%	
Argentina	94	14.9	12.8	Gravina et al. 2010
Argentina	46	10.9	13.0	Dalamón et al. 2005
Brazil	77	3.9	7.8	Cordeiro-Silva et al. 2011
Brazil	33	15.2	12.1	Piatto et al. 2004
Colombia	112	8.9	15.2	Tamayo et al. 2009
Mexico	76	2.6	7.9	Arenas-Sordo et al. 2012
Venezuela	40	5.0	22.5	Utrera et al. 2007
Chile	113	5.3	14.1	This study

N= absence of 35delG

REFERENCES

ABE S, USAMI S, SHINKAWA H, Kelley PM, Kimberling WJ (2000) Prevalent connexin 26 gene (GJB2) mutations in Japanese. J Med Genet 37: 41-43.

ÁLVAREZ A, DEL CASTILLO I, VILLAMAR M, AGUIRRE LA, GONZÁLEZ-NEIRA A, LÓPEZ-NEVOT A, MORENO-PELAYO MA, MORENO F (2005) High prevalence of the W24X mutation in the gene encoding connexin-26 (GJB2) in Spanish Romani (gypsies) with autosomal recessive non-syndromic hearing loss. Am J Med Genet 137A: 255-258.

ARANCIBIA M, RAMÍREZ R, FARFÁN C, ACUÑA M, CIFUENTES L (2012) Frequency of the 35delG mutation of GJB2 gene (connexin 26) in a sample of deaf school children in Santiago. Rev Chil Otorrinolaringol Cir Cabeza Cuello 72: 7-14.

ARENAS-SORDO M DE L, MENÉNDEZ I, HERNÁNDEZ-ZAMORA E, SIRMACI A, GUTIÉRREZ-TINAJERO D, McGETRICK M, MURPHY-RUIZ P, LEYVA-JUÁREZ X, HUESCA-HERNÁNDEZ F, DOMÍNGUEZ-AGURTO J, TEKIN M (2012) Unique spectrum of GJB2 mutations in Mexico. Int J Pediatr Otorhinolaryngol 76: 1678-1680.

BATISSOCO AC, ABREU-SILVA RS, BRAGA MC, LEZIROVITZ K, DELLA-ROSA V, ALFREDO T JR, OTTO PA, MINGRONI-NETTO RC (2009) Prevalence of GJB2 (connexin-26) and GJB6 (connexin-30) mutations in a cohort of 300 Brazilian hearing-impaired individuals: implications for diagnosis and genetic counseling. Ear Hear, 30: 1-7.

BROBBY GW, MULLER-MYHSOK B, HORSTMANN RD (1998) Connexin 26 R143W mutation associated with recessive non-syndromic sensorineural deafness in Africa. N Engl J Med 338: 548-550.

CIFUENTES L, MORALES, SEPÚLVEDA D, JORQUERA H, ACUÑA M (2004) DYS19 and DYS199 loci in a Chilean population of mixed ancestry. Am J Phys Anthropol 125: 85-89.

CORDEIRO-SILVA M DE F, BARBOSA A, SANTIAGO M, PROVETTI M, DETTOGNI RS, TOVAR TT, RABBI-BORTOLINI E, LOURO ID (2011) Mutation analysis of GJB2 and GJB6 genes in Southeastern Brazilians with hereditary non-syndromic deafness. Mol Biol Rep 38: 1309-1313.

CRYNS K, ORZAN E, MURGIA A, HUYGEN PL, MORENO F, DEL CASTILLO I, CHAMBERLIN GP, AZAIEZ H, PRASAD S, CUCCI RA, LEONARDI E, SNOECKX RL, GOVAERTS PJ, VAN DE HEYNING PH, VAN DE HEYNING CM, SMITH RJ, VAN CAMP G (2004) A genotype-phenotype correlation for GJB2 (connexin 26) deafness. J Med Genet 41: 147-154.

DALAMÓN V, BÉHÈRAN A, DIAMANTE F, PALLARES N, DIAMANTE V, ELGOYHEN AB (2005) Prevalence of GJB2 mutations and the del(GJB6-D13S1830) in Argentinean non-syndromic deaf patients. Hear Res 207: 43-49.

DEL CASTILLO I, VILLAMAR M, MORENO-PELAYO MA, DEL CASTILLO FJ, ÁLVAREZ A, TELLERIA D, MENÉNDEZ I, MORENO F (2002) A deletion involving the connexin 30 gene in non syndromic hearing impairment. N Eng J Med 346: 243-249

DEL CASTILLO FJ, RODRÍGUEZ-BALLESTERS M, ÁLVAREZ A, HUTCHIN T, LEONARDI E, DE OLIVEIRA CA, AZAIEZ H, BROWNSTEIN Z, AVENARIUS MR, MARLIN S, PANDYA A, SHAHIN H, SIEMERING KR, WEIL D, WUYTS W, AGUIRRE LA, MARTÍN Y, MORENO-PELAYO MA, VILLAMAR M, AVRAHAM KB, DAHL HH, KANAAN M, NANCE WE, PETIT C, SMITH RJ, VAN CAMP G, SARTORATO EL, MURGIA A, MORENO F, DEL CASTILLO I (2005) A novel deletion involving connexin-30 gene, del (GJB6-D13S1854), found in trans with mutation in the GJB2 gene (connexin-26) in subjects with DFNB1 non-syndromic hearing impairment. J Med Genet 42: 588-594.

DENOYELLE F, MARLIN S, WEIL D, MOATTI L, CHAUVIN P, GARABÉDIAN (1999) Clinical features of the prevalent form of childhood deafness, DFNB1, due to a connexi-26 gene defect: implications for genetic counselling. Lancet 353: 1298-1303.

ESTIVILL X, FORTINA P, SURREY S, RABIONET R, MELCHIONDA S, D'AGRUMA L, MANSFIELD E, RAPPAPORT E, GOVEA N, MILÀ M, ZELANTE L, GASPARINI P (1998) Connexin-26 mutations in sporadic and inherited sensorineural defness. Lancet 351 : 394-398.

GRAVINA LP, FONCUBERTA RC, PRIETO ME, GARRIDO J, BARREIRO C, CHERTKOFF L (2010) Prevalence of DFNB1 mutations in Argentinean children with non-syndromic deafness. Report os a novel mutation I GJB2population. Impact on the newborn hearing screening. Int J Pediatr Otorhinolaryngol 74: 250-254.

KENNESON A, VAN NARDEEN BRAUN, K, BOYLE C (2002) GJB2 (connexin 26) variants and nonsyndromic sensorineural hearing loss: A HuGE review. Genet Med 4: 258-274.

KUDO T, IKEDA K, KURE S, MATSUBARA Y, OSHIMA T, WATANABE KI, KAWASE T, NARISAWA K, TAKASAKA T (2000) Novel mutations in the connexin 26 gene (GJB2) responsible for childhood deafness in the Japanese population. Am J Hum Genet 90: 141-145.

LÓPEZ G, PUGA A, PITTALUGA E, BUSTAMANTE M, GODOY C, REPETTO MG (2012) Evaluación de mutaciones en los genes GJB2 y GJB6 en pacientes con sordera congénita identificados mediante *screening* neonatal. Rev Chil Pediatr 83: 154-160.

MARAZITA ML, PLOUGHMAN LM, RAWLINGS B, REMINGTON E, ARNOS KS, NANCE WE (1993) Genetic epidemiological studies of early-onset deafness in the U.S. school-age population. Am J Med Genet 46: 486-491.

MILLER SA, DYKES D, POLESKY HA (1998) Simple salting out procedure for extracting DNA from Human nucleated cells. Nucleids Acid Res 16: 1215.

MORTON NE. (1991) Genetic Epidemiology of hearing impairment. Ann N Y Acad Sci 630: 16-31.

NAZAR M, GOYCOLEA M, GODOY JM, RIED E, SIERRA M (2009) Evaluación auditiva neonatal universal: Revisión de 10 000 pacientes estudiados. Rev. Otorrinolaringol Cir Cabeza y Cuello 69: 93-102.

NIVOLINI K, SILVA-COSTA S, POMÍLIO MCA, PEREIRA T, LOPES KDE C, DE MORAES VC, ALEXANDRINO F, DE OLIVEIRA CA, SARTORATO EL (2010) Newborn hearing screening and genetic testing in 8974 Brazilian neonates. Int J Pediatr Otorhinolaryngol 74: 926-929.

PARK HJ, HAHN SH, CHUN YM, PARK K, KIM HN (2000) Connexin-26 mutations associated with nonsyndromic hearing loss. Laryngoscope 110: 1535-1538.

PETIT C, LEVILLIERS J, HARDELIN JP (2001) Molecular genetics of hearing loss. Annu Rev Genet 35: 589-646.

PIATTO VB, BERTOLLO EMG, SARTORATO EL, MANIGLIA JV (2004) Prevalence of the GJB2 mutations and the del(GJB6-D13S1830) mutation in Brazilian patients with deafness. Her Res 196: 87-93.

SCOTT D, KRAFT M, CARMI R, RAMESH A, ELBEDOUR K, YAIRI Y, SRISAILAPATHY CR, ROSENGREN SS, MARKHAM AF, MUELLER RF, LENCH NJ, VAN CAMP G, SMITH RJ, SHEFFIELD VC (1998) Identification of mutations in the connexin 26 gene that cause autosomal recessive nonsyndromic hearing loss. Hum Mut 11: 387-394.

The Connexin-deafness homepage. http://davinci.crg.es/deafness/).

The Hereditary hearing loss home page; http://hereditaryhearingloss.org

TAMAYO ML, OLARTE M, GELVEZ N, GÓMEZ M, FRÍAS JL, BERNAL JE, FLÓREZ S, MEDINA D (2009) Molecular studies in the GJB2 gene (Cx26) among a deaf population from Bogotá, Colombia: Results of a screening program. Int J Pediatr Otorhinolaryngol 73: 97-101.

UTRERA R, RIDAURA V, RODRÍGUEZ Y, ROJAS MJ, MAGO L, ANGELI S, HENRÍQUEZ O (2007) Detection of the c.35delG/GJB2 and del(GJB6/D13S1830) mutations in Venezuelan patients with autosomal recessive nonsyndromic hearing loss. Genet Test 11: 347-352.

VALENZUELA CY, ACUÑA M, HARB Z (1987) Gradiente sociogenético en la población chilena. Rev Méd Chile 115: 295-929.

WU BL, KENNA M, LIP V, IRONS M, PLATT O. (2003) Use of a multiplex PCR/sequencing strategy to detect both connexin 30 (GJB6) 342 kb deletion and connexin 26 (GJB2) mutations in cases of childhood deafness. Am J Med Genet 121A(2): 102-108.

ZELANTE I, GASPARINI P, ESTIVILL X, MELCHIONDA S, D'AGRUMA L, GOVEA N, MILÁ M, MONICA MD, LUTFI J, SHOHAT M, MANSFIELD E, DELGROSSO K, RAPPAPORT E, SURREY S, FORTINA P (1997) Connexin 26 mutations associated with the most common form of non-syndromic neurosensory autosomal recessive deafness (DFNB1) in Mediterraneans. Hum Mol Genet 6: 1605-1609.