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ORIGINAL INVESTIGATION

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# The prevalence and expression of inherited connexin 26 mutations associated with nonsyndromic hearing loss in the Israeli population

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**Abstract** Connexin 26 (*GJB2*) mutations lead to hearing loss in a significant proportion of all populations studied so far, despite the fact that at least 50 other genes are also associated with hearing loss. The entire coding region of connexin 26 was sequenced in 75 hearing impaired children and adults in Israel in order to determine the percentage of hearing loss attributed to connexin 26 and the types of mutations in this population. Age of onset in the screened population was both prelingual and postlingual, with hearing loss ranging from moderate to profound. Almost 39% of all persons tested harbored *GJB2* mutations, the majority of which were 35delG and 167delT mutations. A novel mutation, involving both a deletion and insertion, 51del12insA, was identified in a family originating from Uzbekistan. Several parameters were examined to establish whether genotype-phenotype correlations exist, including age of onset, severity of hearing loss and audiological characteristics, including pure-tone audiometry, tympanometry, auditory brainstem response (ABR), and transient evoked otoacoustic emissions (TEOAE). All *GJB2* mutations were associated with prelingual hearing loss, though severity ranged from moderate to profound, with variability even among hearing impaired siblings. We have not found a significant difference in hearing levels between individuals with 35delG and 167delT mutations. Our results suggest that, in Israel, clinicians should first screen for the common 167delT and 35delG mutations by simple and inexpensive restriction enzyme analysis, although if these are not found, sequencing should be done to rule out additional mutations due to the ethnic diversity in this region.

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## Introduction

Knowledge about the molecular basis of deafness is growing rapidly with the identification of thirteen nuclear and two mitochondrial genes involved in nonsyndromic hearing loss (NSHL) (Griffith and Friedman 1999; Van Camp and Smith 1999). Over 100 genes may be involved in NSHL, but the most dramatic recent discovery has been the high incidence of mutations found in the gap junction protein, connexin 26 (locus designation, *GJB2*). Connexin 26 is a member of a large family of proteins that form gap junctions in virtually every cell type. These junctions, composed of multimeric connexons, allow molecules to pass from cell to cell. The connexons are composed of connexins, which vary in their gating properties and cell specificity (Kumar and Gilula 1996). Connexin 26 is highly expressed in the mammalian cochlea, a specialized structure composed of several cell types, including neurons, epithelial cells, and connective tissue, all requiring cell-to-cell communication (Kikuchi et al. 1995; Kelsell et al. 1997). Though their role in the cochlea has not been elucidated completely, gap junctions are thought to allow $K^+$, whose flow generates receptor potentials of hair cells, to recirculate back into the endolymph (Spicer and Schulte 1996).

The *DFNB1* locus, which is located on chromosome 13q11–12, was the first deafness recessive locus to be discovered. *DFNB1* was initially identified by linkage analy-

sis in a large Tunisian family exhibiting recessive hearing loss (Guilford et al. 1994). Genotyping of additional families eventually led to the identification of many more *DFNB1* families (Maw et al. 1995), and the high prevalence of mutations at this locus became apparent once mutations in the connexin 26 gene were identified (Kelsell et al. 1997). The increase in the number of connexin 26 mutations identified was facilitated by the size of this small gene, composed of only one coding exon. In some geographical regions, as many as 50% of hearing impaired individuals harbor mutations in this small gene (Denoyelle et al. 1997). One mutation, 35delG (also referred to as 30delG), accounts for the majority of mutant alleles. The 167delT mutation is the second most prevalent *GJB2* mutation and, so far, has only been found in the Ashkenazi Jewish population (Kelley et al. 1998; Morell et al. 1998; Cohn et al. 1999). High carrier frequency of this mutation has also been found in the Ashkenazi hearing population (Morell et al. 1998; Sobe et al. 1999). Although most connexin 26 mutations are recessive, a mutation has been identified in dominantly inherited hearing loss as well (Denoyelle et al. 1998).

The geographical distribution and demographic changes in the Israeli population over the last 50 years have led to a population of diverse ethnic groups, derived from Europe, America, Asia and Africa (DellaPergola 1992; Bonne-Tamir et al. 1992). Today, this population is represented by approximately 5.7 million people, comprising 4.6 million Jews and 1.1 million Arabs (Central Bureau of Statistics, Israel; http://www.cbs.gov.il/israel_in_figures/population.htm). The precise number of cases of profound early-onset NSHL in Israel is unknown, but it is expected to be similar to that of the Western world, namely affecting approximately 1 in 1000 children (Marazita et al. 1993). This rate is higher in the Israeli Arab population due to consanguinity in Arab villages (Jaber et al. 1998). The first mutation associated with nonsyndromic hearing loss, the A1555G mutation in the 12S ribosomal RNA mitochondrial gene, was found in an Israeli Arab extended family (Prezant et al. 1993). The *DFNA15* locus was identified in a large Israeli Jewish family exhibiting autosomal dominant progressive NSHL (Vahava et al. 1998). One of the first reports on connexin 26 mutations involved a study of two large Muslim Israeli Arab kindreds (Carrasquillo et al. 1997), and a report describing the prevalence of connexin 26 mutations in Mediterranean populations included Israeli Jewish and Israeli Arab individuals (Zelante et al. 1997). Early estimates predicted that there are eight or nine deafness genes in the Israeli population (Brownstein et al. 1991), but based on recent worldwide mutation screens, we anticipated that a significant proportion of NSHL will be due to connexin 26 mutations.

As part of a study to determine the prevalence and expression of connexin 26 mutations in the Israeli nonsyndromic, hearing impaired population, a total of 75 hearing impaired individuals (61.3% with a family history of NSHL and 38.7% with sporadic sensorineural hearing loss) were examined for mutations in the *GJB2* gene. An examination of pure-tone audiometry, otoacoustic emissions and auditory brainstem response (ABR) testing from a subset of individuals was analyzed to determine whether there is a correlation of phenotype with mutations in connexin 26. Furthermore, this study addresses whether only a select group of connexin 26 mutations should be screened for in the Israeli hearing impaired population, or whether the entire coding region of the gene should be examined, with implications for genetic counseling.

## Subjects and methods

### Family data

Individuals included in this study with a family history of NSHL had at least two hearing impaired individuals in the immediate or extended family. Individuals with sporadic cases of NSHL did not report any known hearing loss in their families. A complete clinical history of each affected individual was collected to ensure that the hearing loss was not a result of infection, trauma, acoustic trauma and/or ototoxic drugs, rubella or premature birth. This project was approved by Helsinki Committees at Tel Aviv University, Tel Aviv Sourasky Medical Center, Haim Sheba Medical Center, and Rabin Medical Center. Blood samples and buccal smears were obtained following informed consent from each individual and, in the case of individuals under 18, from their parents.

### Audiology

Hearing of most probands was measured by pure-tone audiometry at the MICHA Society for Hearing Impaired Children, certified by Tel Aviv University for clinical supervision, and at other centers where audiology is performed. Severity of hearing impairment was classified as follows: <25 dB, normal hearing; 25–44 dB, mild hearing loss; 45–64 dB, moderate hearing loss; 65–84 dB, severe hearing loss; and >85 dB, profound hearing loss. The degree of loss was classified by averaging frequencies at 500, 1000, and 2000 Hz according to the better ear. The hearing loss was sensorineural in all cases and bilateral in all but one individual examined. Tympanometry, ABR and transient evoked otoacoustic emissions (TEOAE) were done in a selected patient population. The audiometric curves for adults were compared with age- and sex-dependent percentile curves (International Organization for Standardization, International Standard ISO 7029 1984).

### Mutation detection

Mutation detection was performed by direct sequencing of PCR products. Mutations in *GJB2* were identified by comparing sequence derived from DNA of unaffected and affected individuals. Genomic DNA was prepared from blood (Miller et al. 1988) or buccal smears (Richards et al. 1993) and amplified by PCR using primers C280 (1F, 5'-TCT TTT CCA GAG CAA ACC GC-3' and 1R, 5'-GAC ACG AAG ATC AGC TGC AG-3') and C500 (2F, 5'-CCA GGC TGC AAG AAC GTG TG-3' and 2R, 5'-GGG CAA TGC GTT AAA CTG GC-3') (Kelsell et al. 1997). A portion of the mutation analysis was performed on a 722 bp PCR product, using the 1F and 2R primers. To amplify the first (noncoding) exon and the flanking donor splicing site, amplification was performed using the Advantage-GC Genomic PCR Kit (Clontech) and PCR primers EXON1 A (5'-TCC GTA ACT TTC CCA GTC TCC GAG GGA AGA GG-3') and EXON1 M (5'-CCC AAG GAC GTG TGT TGG TCC AGC CCC-3') (Denoyelle et al. 1999). The PCR reactions were purified on a High-Pure PCR Product Purification Kit column (Boehringer Mannheim). Sequencing was performed using the same primers as those used for PCR, the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer Applied Biosystems) and an ABI 377 DNA se-

quencer. Mutations were confirmed by sequencing in both directions. To screen for mutations in the acceptor splicing site of coding exon 2 and the entire ORF, primers GAP1F (5'-CCT ATG ACA AAC TAA GTT GGT TC-3') and CONNR (5'-GAC AGC TGA GCA CGG GTT GCC TC-3') were used (Denoyelle et al. 1999).

Restriction enzyme digestions were performed on PCR products in order to detect the 35delG and 167delT mutations. For 35delG, modified primers were designed to include a *Bsl*I site that would appear due to a deletion of a G between nucleotides 30 and 35 (Storm et al. 1999). For the 167delT mutation, a *Pst*I site is lost due to the deletion. A *Pst*I digest of the 722 bp amplified wild-type DNA produced four fragments of 452, 150, 69 and 51 bp; digestion of DNA from homozygotes produced 452, 219 and 51 bp fragments.

### Allele separation of heterozygotes

When a sequence appeared to be an overlap of two different sequences, alleles were separated by subcloning several PCR products into pBluescript using the PCR Script Cloning Kit (Stratagene). Each allele was confirmed after sequencing at least ten clones.

### Haplotype analysis

Genotyping markers from 13q11 were used to examine haplotypes of 35delG and 167delT mutations in the hearing impaired probands and some of their parents. Markers D13S141, D13S250 and D13S175, all within approximately 2 cM of *GJB2*, were used (Carrasquillo et al. 1997). Alleles for each marker are the same as those reported in Morell et al. (1998).

## Results

Sequence analysis of the entire coding region of connexin 26 revealed 29/75 (38.7%) mutations in the population studied (Table 1). Of individuals with a family history of NSHL, 41.3% (19/46) (both prelingual and postlingual) were found to have connexin 26 mutations. Ten out of 29 (34.5%) sporadic cases of NSHL examined were found to have mutations in *GJB2*. Of the 54 mutated chromosomes identified, 31 carry the 35delG mutation, 21 carry the 167delT mutation, and two carry a novel mutation, 51del12insA (Table 2).

### Previously identified mutations

Two mutations found in this study were described previously. Twenty unrelated individuals harbored the common 35delG mutation, in both the homozygous, heterozygous, and compound heterozygous form (Table 2). This 1 bp deletion in a stretch of six G nucleotides, which lies in codon 10, results in a frameshift; a glycine is converted to a valine at codon 12 and a stop codon is formed at codon 13 (Fig. 1). Eleven probands were homozygous for this mutation (B1391, D6, D9, D25, D33, D38, D45, D48, D66, I285, T217) and five were compound heterozygous (D49, D51, D61, I91, I98). In four cases (D37, D46, I2, T273), a second *GJB2* mutation, if one exists at all, was not found in the coding region despite sequencing at least ten clones derived from allele separations. No mutations were found in the noncoding exon 1 and flanking donor splicing site or in the acceptor splicing site of coding exon 2.

The 167delT mutation was detected in thirteen cases of hearing loss, eight in the homozygous form (D10, D40, D55, D56, D60, D65, I29, T99) and five times as compound heterozygotes (D49, D51, D61, I91, I98). The 167delT mutation is the second most common connexin 26 mutation described and has only been identified in the Jewish Ashkenazi population (Kelley et al. 1998; Morell et al. 1998; Sobe et al. 1999). All individuals in this study with 167delT mutations were from Ashkenazi Jewish families. The 167delT deletion leads to a frameshift at codon 56 and premature termination occurs at the end of 25 novel codons (Fig. 1).

### Novel mutation

A novel *GJB2* deletion/insertion was found in the DNA of a child from family R (R1) with a family history of profound NSHL. His two profoundly deaf siblings had the same mutation in connexin 26. The parents are second cousins originating in Samarkand, Uzbekistan. All children exhibit prelingual, bilateral, sensorineural hearing loss, with no other abnormalities. There is one other report of prelingual deafness in the extended family (unavailable for analysis). A deletion of 12 bp occurred, with the insertion of one nucleotide, to form a 51del12insA mutation (Fig. 2). A frameshift is formed in the amino terminal portion of the protein, resulting in 26 additional

**Table 1** Summary of probands

| Hearing loss | Number of individuals | *GJB2* mutations | |
|---|---|---|---|
| | | Homozygous or compound heterozygous | Heterozygous |
| Prelingual hearing loss | | | |
| Familial | 36 | 16 | 3 |
| Sporadic | 28 | 9 | 1 |
| Postlingual hearing loss | | | |
| Familial | 10 | 0 | 0 |
| Sporadic | 1 | 0 | 0 |

**Table 2** *GJB2* mutations and clinical findings (*con* consanguinous)

| Proband | Ethnic origin | Family history of hearing loss | Genotype | Deafness |
|---|---|---|---|---|
| B1391 | Jewish | Yes | 35delG/35delG | Prelingual; severe to profound |
| D6 | Arab | Yes, con | 35delG/35delG | Prelingual |
| D9 | Arab | No, con | 35delG/35delG | Prelingual; profound[1] |
| D25 | Jewish/non-Jewish | No | 35delG/35delG | Prelingual; moderate to severe[1] |
| D33 | Jewish | Yes | 35delG/35delG | Prelingual; profound[1] |
| D38 | Jewish | No | 35delG/35delG | Prelingual; profound[1] |
| D45 | Jewish | Yes | 35delG/35delG | Prelingual; profound |
| D48 | Non-Jewish | Yes | 35delG/35delG | Prelingual; profound[1] |
| D66 | Jewish | Yes | 35delG/35delG | Prelingual |
| I285 | Arab | Yes | 35delG/35delG | Prelingual; profound |
| T217 | Jewish | Yes | 35delG/35delG | Prelingual |
| D37 | Jewish (Ashkenazi) | Yes | 35delG/+[2] | Prelingual; profound[1] |
| D46 | Jewish | Yes | 35delG/+ | Prelingual; moderate to severe[1] |
| I2 | Jewish (Ashkenazi) | No | 35delG/+ | Prelingual; profound[1] |
| T273 | Jewish | Yes | 35delG/+ | Prelingual; profound |
| D49 | Jewish (Ashkenazi) | Yes | 35delG/167delT | Prelingual; moderate to severe[1] |
| D51 | Jewish (part Ashenazi) | No | 35delG/167delT | Prelingual; profound[1] |
| D61 | Jewish (Ashkenazi) | Yes | 35delG/167delT | Prelingual; severe[1] |
| I91 | Jewish (Ashkenazi) | Yes | 35delG/167delT | Prelingual; profound[1], severe[1] (two siblings) |
| I98 | Jewish (Ashkenazi) | No | 35delG/167delT | Prelingual; mild to moderate[1] |
| D10 | Jewish (Ashkenazi) | Yes | 167delT/167delT | Prelingual; profound[1] |
| D40 | Jewish (Ashkenazi) | No | 167delT/167delT | Prelingual; profound[1] |
| D55 | Jewish (Ashkenazi) | No | 167delT/167delT | Prelingual; severe to profound |
| D56 | Jewish (Ashkenazi) | Yes | 167delT/167delT | Prelingual; severe to profound |
| D60 | Jewish (Ashkenazi) | No | 167delT/167delT | Prelingual; profound[1] |
| D65 | Jewish (Ashkenazi) | No | 167delT/167delT | Prelingual; profound[1] |
| I29 | Jewish (Ashkenazi) | Yes | 167delT/167delT | Prelingual; profound |
| T99 | Jewish (Ashkenazi) | Yes | 167delT/167delT | Prelingual; profound[1] (two siblings) |
| R1 | Jewish | Yes, con | 51del12insA/51del12insA | Prelingual; profound[1], severe[1], severe[1] (three siblings) |

[1]See audiograms in Fig. 3. Other audiograms are unavailable, and hearing status is by family report
[2]No coding sequence variant found

novel amino acids followed by a premature termination (Fig. 1). Previously, insertions, missense, and small (1 bp) to large (38 bp) deletions have been found in connexin 26 (Rabionet et al. 1999). This is the first report of both a deletion and insertion appearing simultaneously to form a *GJB2* mutation.

## Haplotype analysis

All but one 167delT homozygote (of four genotyped) had the 3/4 haplotype for the D13S141/D13S175 markers, as was previously shown to be the case for hearing impaired Ashkenazi individuals with the homozygous 167delT mutation and for the majority of carriers (Morell et al. 1998) (Table 3). This exceptional case (I29; found for two hearing impaired siblings) may be due to a historic recombination event occurring proximal to the connexin 26 gene, and/or in the case of D13S250, to slippage of 2 bp between allele 1 and 2. The D13S250 marker is less informative with only two alleles, but five out of eight chromosomes carried the 1 allele. All 35delG homozygotes (nine genotyped) had the 3/4 haplotype as well, so that we are unable to make a conclusion regarding the random or founder effect nature of the 35delG mutation in our population. The D13S250 marker varied, albeit only with two alleles.

## Audiologic characteristics

Hearing patterns were quite variable, even between siblings with the same connexin 26 mutation (Fig. 3). All audiograms showed moderate to severe to profound hearing loss. Tympanometry, which is an indirect measure of the compliance of the tympanic membrane and the ossicular system, when normal in a deaf child, indicates sensorineural hearing loss (Grimes 1998). Tympanometry results in a subset of individuals (D9, D10, D25, D37, D38, D40, D51, I2, I91, I98, R1) indicated proper functioning of the tympanic membrane and middle ear (data not shown). These children were examined periodically and at times were found to be type B due to serous otitis media (SOM). Otoacoustic emissions (OAE) encompass a series of indirect tests, including transient evoked otoacoustic emissions (TEOAE), distortion products (DP) and spontaneous otoacoustic emissions (SOAE), that measure the integrity of outer hair cell function and thus assess cochlear function (Grose and Hall 1998). In all individuals tested

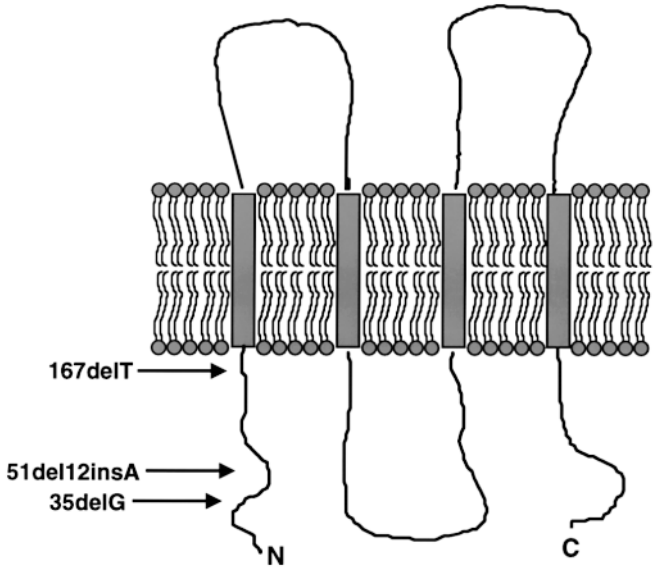


**Fig. 1** Schematic representation of the connexin 26 protein, with *arrows* showing location of mutations found in the Israeli population in this study. The 35delG mutation is commonly found in the rest of the world and is associated with recessively inherited deafness. The 167delT mutation has been found only in the Ashkenazi Jewish population. The 51del12insA mutation has not been found previously. The 35delG mutation forms a stop codon immediately following the mutation. For the 167delT and 51del12insA mutations, translation continues with novel amino acids formed, but the transmembrane domains are presumably not translated in both cases

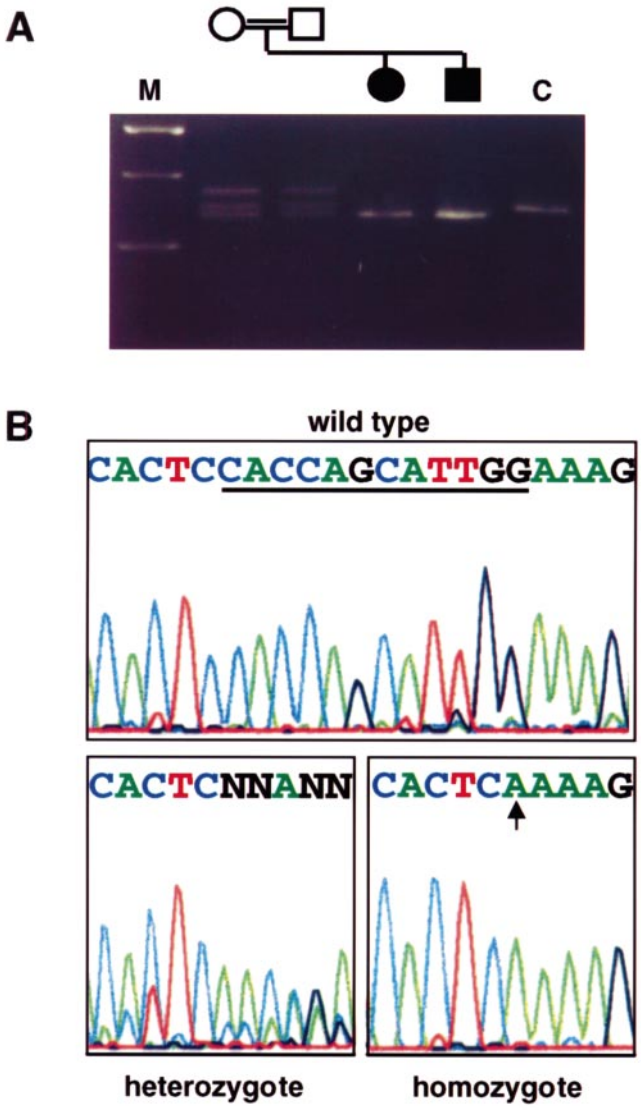


**Fig. 2** Analysis of the novel *GJB2* mutation in family R. **A** PCR amplification of the 280 bp connexin 26 fragment on a 3.5% MetaPhor (FMC BioProducts) gel. *Lower band* shows the homozygote 51del12insA band; the *middle band* is the wild type allele. The *top band* in the parents is a heteroduplex formed between wild type and mutant alleles. **B** Sequence analysis of the novel connexin 26 mutation. The *underlined* 12-bp sequence, shown in the *upper panel*, is deleted in members of family R with NSHL (*lower right panel*). The inserted A is indicated

for TEOAE (D25, I2, R1), there was no response, consistent with a lack of cochlear function due to *GJB2* mutations. Evoked potentials associated with structures in the brainstem are measured as ABR and are a measure of a functional central auditory pathway (Grose and Hall 1998). As part of the assessment of the hearing loss in children, ABRs were performed to confirm results of pure-tone audiometry. In all tested individuals (D9, D10, D25, D37, D38, D40, D51, I2, I91, R1), the results of the ABRs were consistent with the hearing loss measured by pure-tone audiometry.

An examination of relatives of D25 revealed a hearing loss below the 95th percentile (p95) of the standard reference curves (ISO 1984) in both the father and an aunt, although they are both heterozygous 35delG carriers. The father's hearing loss onset was at the age of 30 years, and at the present time, is mild (Fig. 3). The aunt began to lose her hearing during childhood and suffers from high frequency hearing loss.

## Discussion

Our observation, that close to 39% of a subset of the hearing impaired population in Israel have mutations in the connexin 26 gene, is in the range of that reported elsewhere in the world; for example, 49% of cases with recessive deafness and 37% of sporadic cases in Italy and Spain (Estivill et al. 1998); 10% of sporadic cases in the UK and Belgium (Lench et al. 1998); and 40% of cases with recessive deafness in Americans (Kelley et al. 1998). Though the greatest proportion of mutations were the common 35delG and 167delT mutations, one novel mutation was identified in the Israeli population. The 51del12insA mutation occurs in the first intracellular amino terminal domain, leading to a stop codon at the beginning of the first extracellular domain. During DNA transcription, two DNA strands are polymerized at the replication fork, resulting in incorporation of new nucleotides on the new strand. The inappropriate insertion of an extra base, mediated by a stretch of identical bases,

**Table 3** Haplotype of probands with the 35delG and/or 167delT mutations

| Haplotype | D13S250 | D13S175 | *GJB2* | D13S141 | Frequency |
|---|---|---|---|---|---|
| A | 1 | 4 | 35delG | 3 | 12 |
| B | 2 | 4 | 35delG | 3 | 6 |
| A or B | 1 or 2 | 4 | 35delG | 3 | 5 |
| C | 1 | 4 | 167delT | 3 | 5 |
| D | 2 | 4 | 167delT | 3 | 2 |
| C or D | 1 or 2 | 4 | 167delT | 3 | 2 |
| E | 2 | 8 | 167delT | 3 | 1 |
| F | 1 or 2 | 4 | Unknown | 2 | 2 |
| G | 1 or 2 | 5 | Unknown | 4 | 1 |
| Undetermined[1] | 1 or 2 | 3 or 4 | 35delG | 3 or 2 | 2 |
| Undetermined[1] | 1 or 2 | 3 or 4 | 167delT | 3 or 2 | 2 |

[1]Haplotype has not been determined due to lack of parental information

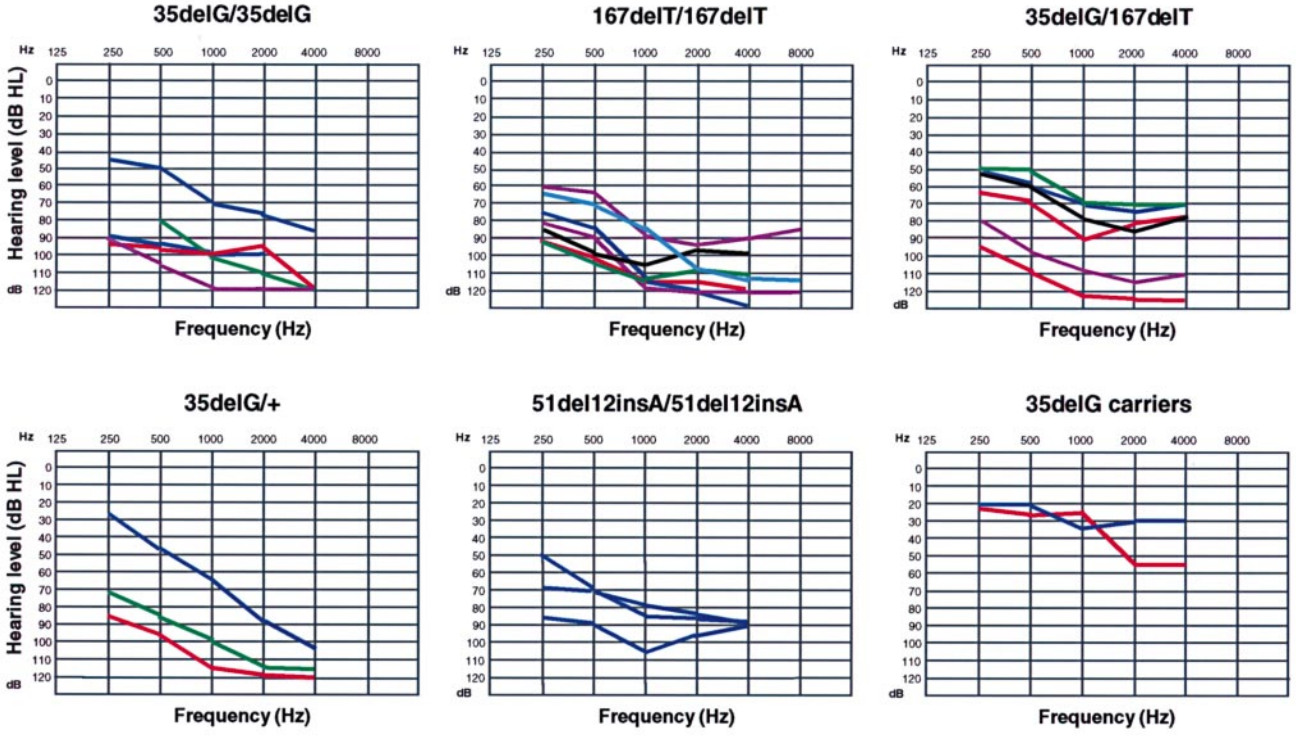


**Fig. 3** A composite of audiograms from individuals, grouped according to their connexin 26 mutations. Only thresholds for the better ear in each case are shown. Siblings are represented by audiometric curves in the same color. 35delG/+ are probands in this study with prelingual hearing loss (D37, D46, I2). 35delG carriers are adult relatives with postlingual onset hearing loss of a 35delG/35delG proband (D25). The *horizontal axis* shows tone frequency (*Hz*) and the *vertical axis* displays hearing level (*dB*)

may be due to slipped mispairing at the replication fork, causing DNA polymerase to misincorporate an extra base (Cooper and Krawczak 1991). A systematic study of insertional mutagenesis in human disease suggested that it is a nonrandom event, dependent on surrounding DNA sequence. The insertion event formed in family R appears to be such a case, since an insertion of an A occurs in a stretch of already existing adenine nucleotides.

Previous reports suggest there is no clear correlation between particular connexin 26 mutations and severity of hearing loss (Kelley et al. 1998). Our study agrees with this finding, since severity of hearing loss is different between probands with the same mutation, even between siblings (Fig. 3). Therefore, as previously suggested, modifiers may determine the level of hearing impairment between individuals.

There is a phenotype-genotype correlation regarding the onset of hearing loss that we do observe in our population. Out of 64 cases of prelingual deafness, 29 individuals had mutations in the *GJB2* gene. However, of the 11 postlingual cases examined, none had a connexin 26 mutation. This variability in hearing loss severity and consistency in onset of hearing loss was recently described in a prospective study of French hearing impaired children (Denoyelle et al. 1999).

The 35delG mutation, occurring in a stretch of six Gs, has been postulated to arise from a reoccurring mutation, due to the highly mutable characteristic of a stretch of six

identical nucleotides (Denoyelle et al. 1998). A definitive conclusion cannot be made regarding the origin of the 35delG mutation in our population; other studies support the fact that the 35delG mutation is a recurring one (Denoyelle et al. 1997; Carrasquillo et al. 1997). The 167delT mutation has been proposed to result from a single origin due to conservation of haplotypes surrounding the mutant allele in the Ashkenazi Jewish population (Morell et al. 1998). Our results suggest a recombination occurred following a founder effect, in addition to potential slippage of alleles (Table 3).

One enigma that remains and poses a dilemma for genetic counseling is the number of hearing impaired individuals with 35delG mutations found on only one allele. In our study, we could only detect one mutation in four individuals with prelingual hearing loss. Several other studies have revealed the same rate of 35delG heterozygotes (Denoyelle et al. 1997). One possibility is that these individuals are carriers for the 35delG mutation, detected in approximately 2.5–4% of the general population in which such a study was performed (Estivill et al. 1998), and that their hearing loss is due to mutations in an entirely different gene.

The relevance of typing connexin 26 mutations has been raised with regards to genetic counseling and identifying genotype-phenotype correlations in hearing impairment. Ideally, one would like to predict which gene might be mutated in a group of nonrelated individuals based on clinical parameters and perform mutation analysis for one or two genes. Today, due to the ease of connexin 26 mutation detection, this is the first gene checked in families presenting with NSHL. Indeed, there does not appear to be a single phenotype for those harboring connexin 26 mutations, although there is a trend toward some parameters. The common denominator is sensorineural bilateral hearing loss. The severity ranges from moderate to severe to profound, though most hearing loss in our study was profound. The age of onset is prelingual in all *GJB2* mutations identified in our study. In cases with a family history of NSHL, all homozygous connexin 26 mutations were consistent with inheritance in a recessive mode. None of the dominantly inherited cases of hearing loss had connexin 26 mutations, but this was not surprising since in the world population this has only been reported in two families (Denoyelle et al. 1998).

This study has direct clinical ramifications for genetic counseling in the hearing impaired population, both in Israel and the rest of the world. We would suggest performing the two simple restriction enzyme assays for the common mutations and, if they should prove to be negative, to amplify the entire ORF in one PCR fragment and sequence with one forward primer. The challenge that remains is to identify the genes impaired in the remaining 46 individuals screened who did not have connexin 26 mutations, representing approximately 60% of the hearing impaired population in Israel. This remains difficult since the other mutations do not appear to be in one other specific gene, but may represent several genes. Other than the *POU3F4* (*DFN3* X-linked, and therefore rare), *POU4F3* (*DFNA15*), connexin 31 (*DFNA2*) and *KCNQ4* (*DFNA2*) genes, other NSHL genes identified so far are quite large, hampering mutation screening. Though three of these genes have only been found in dominantly inherited hearing loss so far, the fact that myosin VIIA (*MYO7A*) and *GJB2* mutations have been found associated with both recessive and dominant hearing loss makes these genes candidates for recessive hearing loss as well (Kelsell et al. 1997; Liu et al. 1997a; Liu et al. 1997b; Denoyelle et al. 1998). Possible screening schemes include searching for shared haplotypes when two hearing impaired siblings are available. Despite these current limitations, the ease with which *GJB2* mutations can be detected and the significant proportion of connexin 26 mutations for prelingual moderate to profound NSHL has changed the face of genetic counseling for the hearing impaired.

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