Determination of the frequency of connexin26 mutations in inherited sensorineural deafness and carrier rates in the Tunisian population using DGGE

**EDITOR—**Congenital deafness occurs in approximately 1 in 1000 live births and at least 50% of these cases are hereditary. Among the prelingual genetic forms of deafness, the autosomal recessive forms (DFNB) are frequent (80% of the cases) and in most cases are sensorineural and severe. Twenty eight loci that cause autosomal recessive non-syndromic hearing loss (ARNSHL) have been identified (http://dnalab-www.uia.ac.be./dnalab/hhh/index.html). The first locus defined for recessive deafness (DFNB1) is linked to chromosome 13q12-13 and was identified by homozygosity mapping in two large consanguineous families from Tunisia. This initial report was followed by the identification of several other consanguineous families of different ethnic origins which were linked to the DFNB1 locus and of several non-consanguineous white families in which the ARNSHL phenotype cosegregated with markers from chromosome 13q12-13. Mutations in connexin26 (Cx26), a gene that encodes gap junction protein beta-2 (GJB2), have been shown to result in autosomal recessive (DFNB1) and dominant (DFNA3) non-syndromic sensorineural deafness. Mutations in the Cx26 gene have been found to be the most common cause of autosomal recessive deafness and the most frequently observed mutation is 35delG.

The high prevalence of Cx26 mutations and their importance as a cause of ARNSHL have prompted the development of several different mutation detection assays to screen the single Cx26 coding exon. A rapid method to detect mutations in the GJB2 gene would be very useful in the diagnosis of deafness and in the assessment of carrier status. In this paper, we have developed a denaturing gradient gel electrophoresis (DGGE) method for screening sequence variation in the coding region of Cx26. This technique was used to determine the carrier frequency of the 35delG mutation in 236 unrelated, unaffected Tunisians and to estimate the prevalence of Cx26 mutations in 70 families affected by ARNSHL with various degrees of hearing loss (42 profound, 20 severe, five moderate, three mild). All families were unrelated and originated from different regions of Tunisia.

Two segments of Cx26 gene DNA, each containing a single uniform melting domain, were selected using the computer program MELT94 (http://web.mit.edu/osp/www/melt.html). The segments are defined by the PCR primers Cx175-F/Pso-Cx478-R (TTC CAG AGC AAA CCG CCC AGC psoralenTA-TCC GGT AGG CCA GTG GCA TG) and Cx462-F/Cx780-R (CAG CGG TCC TAG TGG CCA TG psoralenTA-AGA CAC TGC AT CAT GAA CA). PCR amplification was carried out under standard conditions. Temperature cycling for amplification was as follows: 94°C for 40 seconds, 64°C for 30 seconds, and 72°C for 45 seconds for 35 cycles. PCR products of affected and control subjects with and without PCR product of wild type DNA were denatured at 95°C for three minutes and cooled progressively for 30 minutes to 37°C. For psoralen crosslinking, samples were placed on an ELISA plate and exposed to UV light (365 nm) for 18 minutes. Under these conditions, 70-90% of the strands in the reannealed fragments became crosslinked. After crosslinking, the PCR samples were subjected to electrophoresis at 97 V on a 6% polyacrylamide gel with a linear 30-80% denaturant gradient parallel to the direction of electrophoresis. The appropriate running time for DGGE was 16 hours. The gels were stained for 10 minutes with ethidium bromide (1 µg/ml) and photographed with a UV transilluminator. PCR products that showed shifts after DGGE analysis were sequenced on an ABI 377 Perkin Elmer sequencer. The sequencing primers were Cx26A-U/Cx26A-L. The use of a broad gradient of 30-80% denaturant permits simultaneous analysis of a large number of mutations. Psoralen oligonucleotide conjugates provide a good alternative to the commonly used GC tailed oligonucleotides, offering the advantage of lack of strand separation of high denaturant concentration.

DGGE analysis showed two different patterns in affected subjects (fig 1). To confirm the presence and identity of the mutations, PCR product which showed shifts after DGGE analysis were sequenced and the results were confirmed by sequence analysis. In fact, two mutations were

![Figure 1](http://jmedgenet.com/cgi/content/full/37/11/e39)

**Figure 1** Parallel denaturing gradient gel electrophoresis of fragment 1 of the connexin26 gene. (1) Crosslinked wild type (WT), (2) crosslinked mutant type (MT) (homozygous 35delG), (3) crosslinked WT × MT (homozygous 35delG), (4) crosslinked WT × MT (homozygous E47X), (5) crosslinked MT (homozygous delG15) × MT (homozygous E47X), crosslinked MT (heterozygous 35delG). The crosslinked samples of WT and MT gave single bands with nearly identical retardation level. For the crosslinked WT × MT (35delG), the typical heteroduplex-homoduplex doublet can be seen and the two individual heteroduplex bands and the two homoduplex bands are resolved. The crosslinked WT × MT (E47X) sample exhibited a band with the same retardation level, consisting of the two expected types of unresolved homoduplex molecules and a more retarded doublet of bands, consisting of the two expected types of heteroduplex molecules.
detected (35delG and E47X). Homozygous 35delG and E47X mutations were found in 10 and one families, respectively, and we also found E47X in trans with 35delG in another family. A heterozygous 35delG mutation was detected in three controls. Previous indications that the \( DFNB1 \) locus might be a relatively important contributor to recessive prelingual deafness in the Tunisian population\(^1\) were confirmed here; \( Cx26 \) mutations were found in 12 (17%) of the 70 families. The prevalence of the \( Cx26 \) mutation has been shown to vary from 13% in the Japanese population\(^17\) to 4% in subjects from Italy.\(^10 12\)

In our study, the most common \( Cx26 \) mutation found was 35delG. Analysis of 236 unrelated Tunisian people from the general population showed three 35delG heterozygotes, which gave a carrier prevalence of 1.3%. Carrier frequencies for 35delG have been shown to vary from 0.73% in the Ashkenazi Jewish population (where 167delT is seen at a frequency of about 4%) to 4% in subjects from Italy.\(^10 12\)

Recent studies have shown that hearing loss may be mild, moderate, severe, or profound in patients with \( Cx26 \).\(^17 19\) In our study, all patients with \( Cx26 \) mutations have profound bilateral deafness, except in one family where patients were homozygous for the 35delG mutation and exhibited intrafamilial variation in the severity of deafness. This suggests the existence of other factors moderating the expression of the mutant gene.

In conclusion, we have developed a DGGE method for the detection of carriers and affected subjects in deaf families carrying \( Cx26 \) mutations. Our study showed that the prevalence of mutations in the \( Cx26 \) gene was relatively high in the Tunisian population and the most common \( Cx26 \) mutation found was 35delG.

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