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

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 AGG/poralenTA-TCC GGT AGG CCA GTG GCA TG) and Cx462-F/Cx780-R (CAG CGC TCC TAG TGG CCA TG/poralenTA-AGA CAC TGC AAT 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 90 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
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 (17%) of the 70 families. The prevalence of the carrier frequency of the common GJB2 (connexin-26) 35delG mutation in 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 16delTT is seen at a frequency of about 4%) to 4% in subjects from Italy.\(^10\)\(^\text{12}\)

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 16delTT is seen at a frequency of about 4%) to 4% in subjects from Italy.\(^10\)\(^\text{12}\)

Recent studies have shown that hearing loss may be mild, moderate, severe, or profound in patients with Cx26.\(^17\)\(^\text{19}\) 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 modulating 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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