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 nonsyndromic 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 13q12-13.3–6 and was identified by homozygosity mapping in two large consanguineous families from Tunisia. This initial report was followed by the identification of 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 13q12-13.3–6. Mutations in connexin26 (Cx26), a gene that encodes gap junction protein beta-2 (GJB-2), have been shown to result in autosomal recessive (DFNB1) and dominant (DFNA3) non-syndromic sensorineural deafness.7 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.8–11 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.13–16 A rapid method to detect mutations in the GJB2 gene was developed by the use of a broad gradient of 30–80% denaturant in DGGE analysis which permits simultaneous analysis of a large numbers 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** 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 delG35 × MT (homozygous E47X), (6) 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. The crosslinked MT (35delG) × MT (E47X) showed a different pattern in comparison with that obtained with the crosslinked WT × MT (35delG) or the crosslinked WT × MT (E47X).
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 population1 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 population1 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 12delT 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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