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Identification of a locus on chromosome 7q31, DFNB14, responsible for prelingual sensorineural non-syndromic deafness

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In our efforts to identify new loci responsible for non-syndromic autosomal recessive forms of deafness, DFNB loci, we have pursued the analysis of large consanguineous affected families living in geographically isolated areas. Here, we report on the study of a Lebanese family affected with a prelingual profound sensorineural isolated form of deafness. Segregation analysis resulted in a linkage with locus D7S554 to locus D7S2459 on 7q31, with a maximum lod score of 6.3. The causative gene was mapped to a 15 cM interval extending from D7S527 to D7S3074 (on the telomeric side). The distal limit of this interval could be located between D7S496 and D7S3074 which are the closest polymorphic loci flanking the gene underlying Pendred syndrome (*PDS*) on the centromeric and on the telomeric sides, respectively. To eliminate *PDS* as a candidate gene, its 21 exons were sequenced. No mutation was detected. This study therefore reports the identification of a novel locus, DFNB14, on chromosome 7q31, in a position proximal to *PDS*

Keywords: non-syndromic recessive sensorineural hearing loss; homozygosity mapping; Middle East population

Introduction

Most cases of inherited prelingual deafness are isolated or non-syndromic, ie not associated with other abnormalities. Approximately 80% of them are transmitted in an autosomal recessive mode. These forms most frequently cause severe or profound hearing loss, which impede language acquisition. They are genetically highly heterogeneous monogenic diseases.[1] To date, 16 independent loci for the autosomal recessive forms of deafness (DFNB1–3, 5–7/11, 8/10, 9, 12, 13, and 15–19,[2] have been identified. The mapping data was mainly obtained from the study of large consanguineous families living in geographically isolated areas.[1]

For four of these loci, the corresponding genes have been isolated, namely connexin26 (*Cx26* also called *GJB2*) for DFNB1,[3] myosinVIIA (*MYO7A*) for DFNB2,[4,5] myosinXV (*MYO15*) for DFNB3[6] and pendrin (*PDS*) for a DFNB locus[7] initially termed DFNB4.[8]

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In order to identify new *DFNB* loci, we have focused our study on affected families living in isolated villages of different countries around the Mediterranean sea, in particular Lebanon. In this country, 17 religious communities have been identified which correspond, to some extent, to different ethnic groups.[9] By studying the affected families from different communities in northern Lebanon, we have already identified three DFNB loci – DFNB9 and DFNB12 in two different Moslem Sunnite families[10,11] and DFNB13 in a Christian Maronite family.[12] Here, we report the identification of DFNB14, segregating in a Muslim Shiite family (family Ch) living in a village in Southern Lebanon.

Results and Discussion

Clinical Examination

In family Ch, three individuals (V-2, V-5, V-7) born of a marriage between two normal hearing second cousins were affected by hearing loss (see Figure 1). They were recognised as deaf during the prelingual period, although audiometric tests were only performed during this genetic study. The child (VI-1), also deaf, was born of normal hearing first cousin parents. Affected and unaffected members of the family (age ranging from 1 to 45 years) underwent a general clinical examination and audiometric tests. For the 1-year-old child (VI-1), audiometric brain response (ABR) was recorded. Pure-tone audiometry with a recording of pure-tone air and bone conduction thresholds was performed for all the other individuals. Air conduction pure-tone average (ACPTA) threshold in the conversational frequencies (0.5, 1 and 2 kHz) was calculated for each deaf ear, and was used to define the severity of the hearing loss according to the better ear: mild (20 dB ACPTA 39 dB), moderate (40 dB ACPTA 69 dB), severe (70 dB ACPTA 89 dB), profound (ACPTA ≥ 90 dB). The four affected individuals were found to be affected by profound bilateral sensorineural deafness. No symptoms or signs indicative of a syndromic form of deafness were noted in this family.

For reasons described below, following linkage analysis, patients were reexamined and particular attention was paid to the detection of a possible *a minima* form of Pendred syndrome, which is frequently overlooked. This syndrome is characterised by congenital sensorineural hearing loss of variable severity, thyroid goitres and variable degrees of hypothyroidism. No goitre could be detected in any of the deaf individuals in family Ch. Serum T3, T4 and thyroid-stimulating

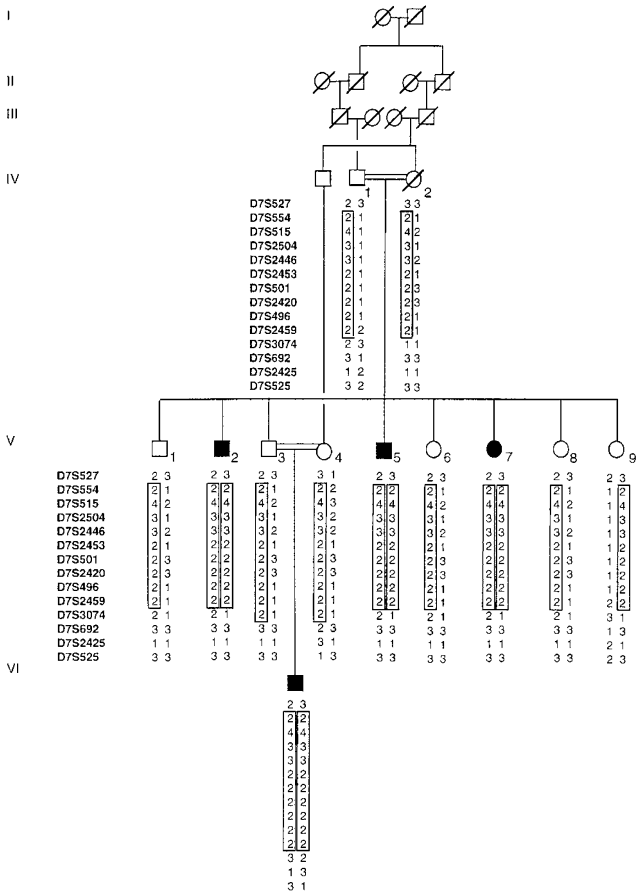

Figure 1 *Segregation analysis of family Ch affected by a sensorineural, isolated, autosomal recessive form of deafness using AFM[18] and sWSS4477 (locus D7S3074)[15] polymorphic markers in the 7q31 region.*
Affected individuals are represented by solid symbols. The core haplotype associated with DFNB14 is boxed. The mother's (IV-2) haplotype has been deduced from her children's haplotypes.

hormone (TSH) levels were normal. In addition, the organification of iodide by the thyroid gland, tested by perchlorate discharge, was examined in the three deaf adults, and found to be normal. Genuine cases of Pendred syndrome with normal perchlorate tests have been reported, although they are rare. Indeed, in a recent study of 13 Pendred affected families,[13] 12 gave abnormal perchlorate discharge test results (ie 14 out of the 16 individuals); in the remaining family, a single deaf individual had been examined. Based on the clinical investigations carried out, including the results of the perchlorate test in three deaf individuals, we could conclude that family Ch is affected by a congenital sensorineural profound autosomal recessive form of non-syndromic deafness.

Linkage Mapping

The possibility that the DFNB locus involved in family Ch was an already defined DFNB locus was first tested. Linkage was found with the 7q31 markers D7S501 and D7S2453, located proximal to *PDS*. A more detailed linkage analysis with microsatellite markers of the region was then undertaken. Linkage was detected with the microsatellite marker D7S515. Analysis of the recombinants showed that the region of homozygosity extended from D7S554 to D7S496; using the MAPMAKER/HOMOZ program, a lod score of 6.3 was calculated. The D7S496 locus has been mapped immediately centromeric (approximately 200 kb) to *PDS*, within a newly identified gene, *NG1*.[14] The closest informative marker which limits the present DFNB candidate gene interval on the distal side is sWSS4477 which defines the D7S3074 locus.[15] The deaf patients V-2, V-5 and V-7 were heterozygous for this marker; it has been positioned approximately 300 kb distal to *PDS*.[15] The only polymorphic marker located between D7S496 and D7S3074, AFMa305ye9 (locus D7S2459), was uninformative. The candidate region thus spans 15 cM, between D7S527 and D7S3074. In one deaf family without thyroid symptoms or signs, but also without a known status defined by the perchlorate test, a missense mutation (affecting a highly conserved glycine residue) has been reported.[7] Therefore, although according to the present mapping data *PDS* was unlikely to be responsible for the hearing loss segregating in family Ch, a search for mutations in this gene was undertaken.

Sequence of the PDS Gene

In two deaf patients (V-2 and V-5) from family Ch, each of the 21 exons of *PDS* and their flanking splicing sites were PCR-amplified, according to Everett *et al*,[15] and sequenced. No mutations were detected. Recently, two independent studies of *PDS* mutations in Pendred affected patients have been reported. One showed that all the expected mutations (ie 23) were detected by sequencing the 21 exons.[13] The second, involving a combination of single strand conformation polymorphism (SSCP) and direct sequencing of 22 affected families with a demonstrated or consistent linkage to the PDS locus, also resulted in the detection of all the expected mutations.[14] Accordingly, the absence of detectable mutations in *PDS* excluded this gene as responsible for the deafness in family Ch.

We thus assume the existence of a gene responsible for a non-syndromic sensorineural autosomal isolated recessive form of deafness corresponding to a novel locus, DFNB14, on 7q31 between D7S527 and *PDS*. Several genes as well as a high number of ESTs have been assigned to the presently defined DFNB14 interval. Before undertaking a systematic analysis of these various genes, one of them, encoding zyxin[16] will be given priority for consideration as a candidate gene. Indeed, zyxin is a cytoskeletal protein implicated in the spatial control of actin filaments assembly, which is likely to be critical for the appropriate organisation and function of the stereociliary bundle of the inner ear hair cells.

Methods

Auditory Tests

Pure tone audiometry with aerial and bone conduction at 250, 500, 1000, 2000, 4000 and 8000 Hz was systematically performed (with a Beltone 2000 clinical audiometer), as well as otoscopic examinations, for each adult individual. In the young affected children, the auditory brainstem response (ABR) was recorded.

Genotyping of Microsatellite Markers

DNA extraction and genotyping were performed as described.[10] The primer sequences of the microsatellite markers have been previously reported,[17] with the exception of D7S3074 reported by Everett *et al*.[15]

Linkage Analysis

Lod scores were calculated using the MAPMAKER/HOMOZ program[18] (version 0.9). The disease was assumed to be inherited in a recessive mode and fully penetrant. The disease allele frequency set at 10^{-3} or 10^{-2} gave lod score values of 6.3 and 5.9, respectively.

Screening for Mutations in the Pendrin Gene

The 21 exons of *PDS* were amplified from genomic DNA, with the primers described elsewhere,[15] and sequenced on ABI 373 or 377 Perkin Elmer sequencers.

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