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A sensorineural progressive autosomal recessive form of isolated deafness, DFNB13, maps to chromosome 7q34-q36

Mirna Mustapha[1], Sébastien Chardenoux[2], Alexandre Nieder[1], Nabiha Salem[1], Jean Weissenbach[3], Elie El-Zir[4], Jacques Loiselet[1] and Christine Petit[2]

[1]Laboratoire de Biochimie, Faculté de Médecine, Université Saint-Joseph, Beyrouth, Lebanon
[2]Unité de Génétique des Déficits Sensoriels, CNRS URA 1968, Institut Pasteur, Paris, France
[3]Centre National de Séquençage, Evry, France
[4]Clinique d'Audiologie, Hôpital du Sacré-Coeur, Baabda, Brazilia, Lebanon

Deafness is the most frequent sensorineural defect in children. The vast majority of the prelingual forms of isolated deafness are highly genetically heterogeneous with an autosomal recessive mode of inheritance. Using linkage analysis, we have mapped the gene responsible for a severe progressive sensorineural hearing loss, DFNB13, segregating in a large consanguineous family living in an isolated region in northern Lebanon. A maximum lod score of 4.5 was detected for markers D7S661–D7S498. Recombination events and homozygosity mapping by descent define a 17 cM gene interval in the chromosome region 7q34-q36, between the markers D7S2468/D7S2505, on the proximal side, and D7S2439, on the distal side.

Keywords: non-syndromic sensorineural deafness; homozygosity mapping; gene localisation

Introduction

The congenital and prelingual inherited forms of isolated deafness are, in 85–90% of the cases, transmitted in an autosomal recessive mode, ([1] for a review). These forms most frequently cause severe or profound hearing loss, which impedes language acquisition. They are genetically monogenic heterogeneous diseases.[2] So far, no clinical signs allow distinction between the defects due to different genes. Due to these characteristics, coupled with the tendency of deaf people to

Correspondence: Dr Christine Petit, Unité de Génétique des Déficits Sensoriels, CNRS URA 1968, Institut Pasteur, 25 rue du Dr Roux, 75724 Paris cedex 15, France. Tel: 33 1 45 68 88 90; Fax: 33 1 45 67 69 78; Email: cpetit@pasteur.fr

intermarry, at least in developed countries, mapping of the corresponding loci (DFNB loci) had long been considered impossible. These difficulties have been circumvented by genetic analysis using three different approaches, ([1] for a review). One of these approaches, the first described in,[1] is based on the study of large affected consanguineous families living in isolated regions for several generations. In such families, the probability of having more than one deafness gene segregating is minimised. Using this approach, we were able to localise the first two genes involved in autosomal recessive deafness, *DFNB1*[3] and *DFNB2*,[4] segregating in large families living in northern and southern Tunisia, respectively. Once linkage to a particular chromosomal region has been detected by linkage analysis, the localisation interval can be refined by the

characterisation of homozygous regions present in affected individuals. To date, 13 loci underlying autosomal recessive forms of deafness have been reported, DFNB1-12 (see[1]) and DFNB16[5] (with four of these probably representing only two different loci, detailed below), mainly by the study of large consanguineous families living in geographic isolates. We have concentrated our search to identify genes responsible for autosomal recessive deafness, by analysing affected families living in isolated villages, on different countries around the Mediterranean Sea, particularly Lebanon.[6,7] In order to identify the target or defective DFNB loci present in the Lebanese population, we have collected blood samples from large affected families from different communities. We report here on the identification of a new locus, DFNB13, in a family belonging to the Christian Maronite faith.

Results

Clinical Description

In family J, a marriage between two first cousins, individuals 40 and 41, resulted in 16 children, 13 of whom are alive (Figure 1). Five of them have been recognised as affected with deafness in the prelingual period, although audiometric tests were only performed during this genetic study. The age of the patients ranged from 8 to 26 years. Pure tone audiometry showed that the youngest individuals were less affected than the older ones. Air and bone conduction revealed a bilateral hearing loss of 50 to 70 dBHL at all frequencies for the younger patients, and 80 to 100 dBHL for the older patients. Audiologic performances of both parents were normal. No other symptom was associated with the deafness, therefore indicating that a progressive, severe form of isolated sensorineural deafness segregates in this family.

Linkage Mapping

In the first instance, we performed an exclusion analysis with the polymorphic microsatellite markers corresponding to nine previously identified DFNB loci, DFNB1,[3] DFNB2,[4] DFNB3,[8] DFNB5,[9] DFNB6,[10] DFNB7/11,[11,12] DFNB8/10,[13,14] DFNB9,[6] DFNB12.[7] We excluded the DFNB4 locus[15] from this analysis, as there is increasing evidence that this locus does not actually underly an isolated form of deafness, but is rather a syndromic form, Pendred syndrome;[16,17] small thyroid goitres have been detected in the older individuals of the previously DFNB4-designated families. Since no

linkage was observed between these nine loci and segregation of the deafness in family J, we undertook an extensive analysis using the Généthon collection of polymorphic markers spaced about 15–20 cM apart.[18] A significant lod score of 4.5 was detected at locus D7S661, on chromosome 7; the five affected children were all homozygous for this marker.

To define further the position of this new locus, DFNB13, the flanking markers were tested (see Figure 2). Analysis of the recombinants in generation IV showed that the affected individual 42 is heterozygous for the most distal locus D7S2439 and that the non-affected individual 49 is homozygous for the three most centromeric loci, D7S684, D7S2505 and D7S2468 (Figure 1). Thereby, the DFNB13 locus was mapped to a 17 cM interval of chromosome 7q34-q36, flanked by D7S2505/D7S2468 (loci not yet ordered) proximally and D7S2439 distally.

Discussion

Using linkage analysis, we have identified a novel locus, DFNB13, underlying an isolated autosomal recessive, progressive form of sensorineural deafness segregating in a large consanguineous family from Lebanon.

Among the 13 DFNB loci identified so far, for only two of them has the corresponding gene been cloned. Mutations in *Cx26*, the gene encoding the gap junction protein connexin 26, have been shown to be responsible for DFNB1 and DFNA3[19–22] and mutations in *MYO7A*, the gene encoding the unconventional myosin VIIA, for DFNB2.[23,24] These two studies also proved correct the hypotheses, based on linkage results, that (*i*) the same gene can be responsible for both a recessive (DFNB1) and a dominant (DFNA3) form of deafness[25] and that (*ii*) the same gene can be responsible for an isolated (DFNB2) and a syndromic (USH1B) form of deafness.[4,26] Since the autosomal dominant form of deafness, DFNA11, has been assigned to the same chromosomal region as DFNB2 and USH1B,[27] *MYO7A* is also likely to underly this dominant form of isolated deafness. Based on these considerations, we searched for a dominant form of isolated deafness or a syndromic form of deafness reportedly assigned to the same chromosomal region as DFNB13. So far, however, neither has been mapped to this chromosomal interval. A locus for the Romano-Ward syndrome, the autosomal dominant form of the long QT syndrome has been assigned to 7q35-q36.[28] The only gene identified as responsible for Jervell and Lange-Nielsen syndrome
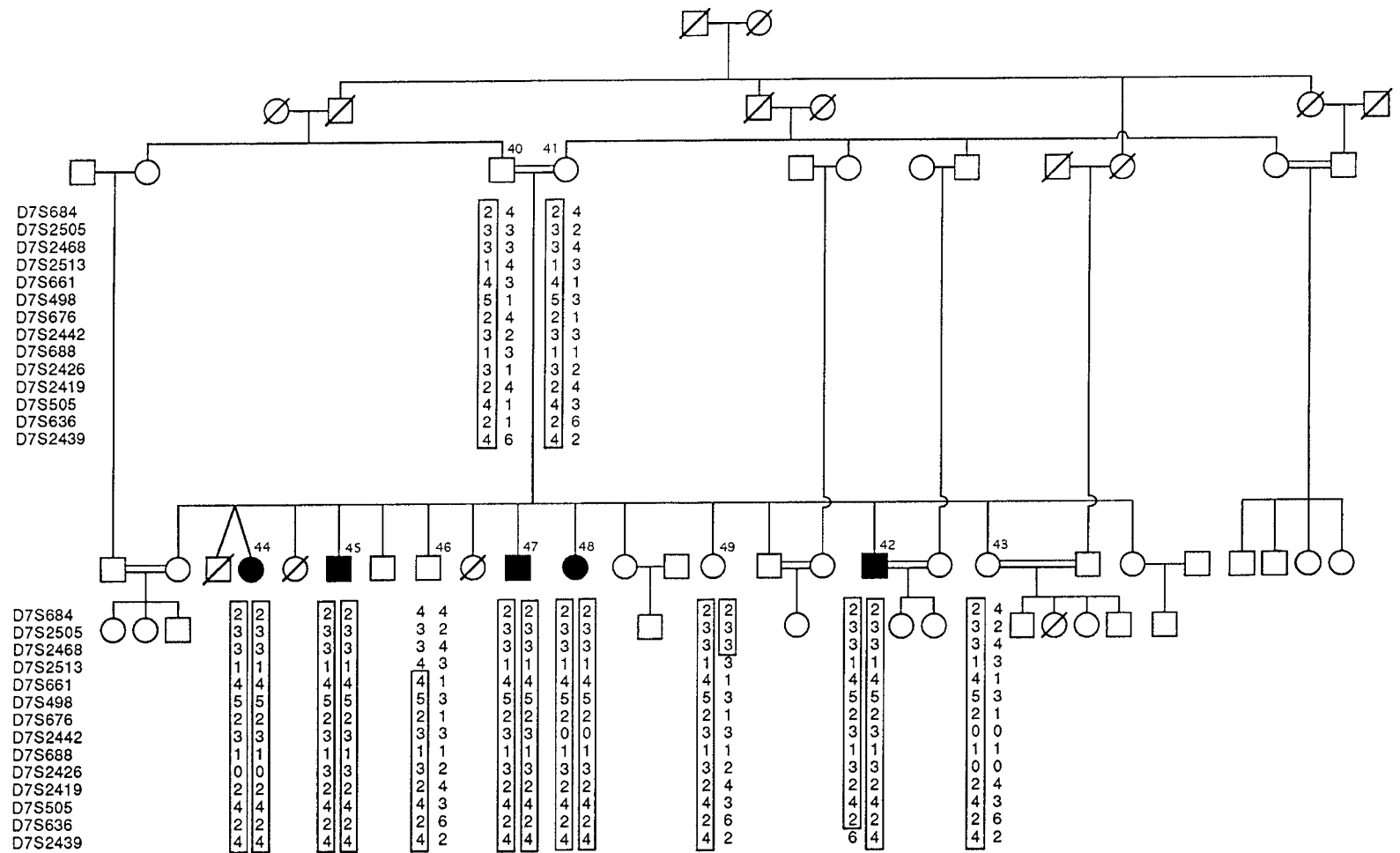
Figure 1 *Segregation analysis of family J affected by a sensorineural, isolated, autosomal recessive form of deafness using AFM polymorphic markers in the 7q34-q36 region.*[18] *Dark symbols represent deaf individuals. The core haplotype associated with DFNB13 is boxed*

(located on 11p15.5), an autosomal recessive disease characterised by a congenital deafness associated with QT prolongation, involves the same gene as one form of Romano-Ward syndrome.[29] Therefore, cardiologic examinations were conducted in family J, the results of which have excluded the possibility that Jervell and Lange-Nielsen syndrome segregates in this family.

Among the numerous deaf mouse mutants, several have already been proven to involve genes orthologous to those implicated in human deafness (for a review, see[1]), therefore facilitating the cloning of the latter. We searched for a deaf mouse mutant assigned to the murine chromosomal region orthologous to human 7q34-q36, ie a region of mouse chromosomes 5 (10–16 cM from the centromere) and 6 (17–20 cM). None has been reported.

Therefore, considering the large size of the chromosomal interval presently defined for *DFNB13*, only a candidate gene approach can be attempted for the

cloning of this gene. A number of ESTs (Expressed Sequence Tags), as well as fully sequenced cDNAs, have been mapped to this chromosomal interval. Among them, priority should be given to the gene encoding the 'single-stranded DNA-binding protein mitochondrial precursor' (SSBP)[30] candidate gene, since mitochondrial metabolism is known to be crucial for auditory function.[31–34] In addition, in order to refine the localisation interval of *DFNB13*, linkage analysis of deaf individuals from additional families living in the same geographical area and belonging to the same community will be performed.
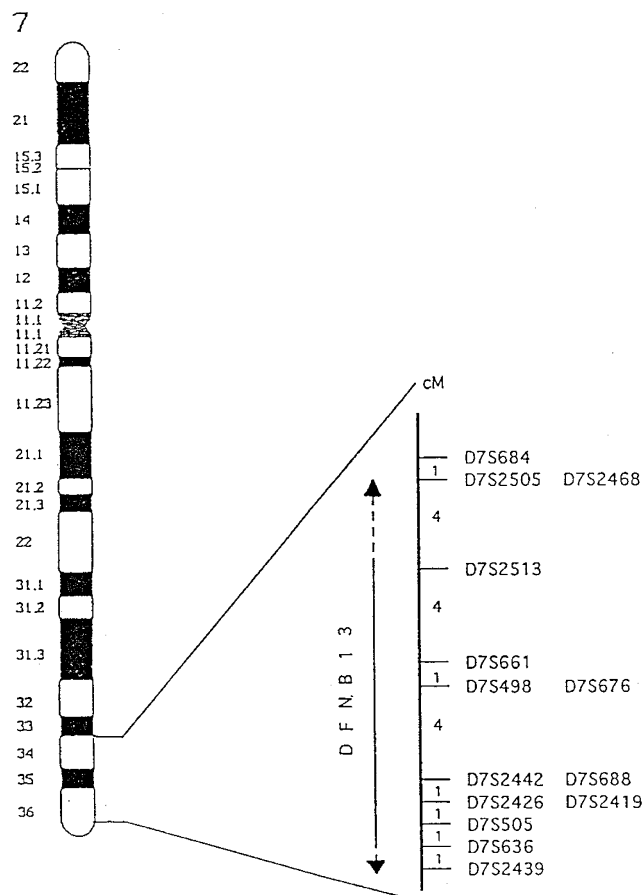

Figure 2 *Schematic representation of chromosome 7 showing the localisation interval of the isolated recessive deafness DFNB13. The indicated loci have been revealed with markers from the Généthon collection[18]*

Materials and Methods

Auditory Tests
Informed consent was obtained from adult subjects and from parents of under-aged patients. Pure tone audiometry with aerial and bone conduction at 250, 500, 1000, 2000, 4000 and 8000 Hz were systematically performed (with Beltone 2000 clinical audiometer), as well as otoscopic examinations, for each individual over 5 years of age.

Genotyping
DNA was prepared from 5–10 ml of blood by standard phenol-chloroform extractions. Fluorescent microsatellite markers[18] were amplified using the polymerase chain reaction (PCR)[35] and resultant products analysed following electrophoresis on a 6% polyacrylamide gel.

Linkage Analysis
Lod scores were calculated using the MAPMAKER/HOMOZ program[36] (version 0.9), an algorithm for rapid multipoint analysis. The disease was assumed to be inherited in a recessive mode and fully penetrant. The gene frequency was set at 10^{-5} and variation of this frequency between 10^{-3} and 10^{-5} resulted in negligible variations of the multipoint lod scores. Meiotic recombination frequencies were assumed to be equal for males and females. The allele frequencies of the polymorphic markers were assumed to be equal.

Acknowledgements

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