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A gene responsible for a sensorineural nonsyndromic recessive deafness maps to chromosome 2p22-23

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The recessive mode of transmission accounts for ~75% of inherited non syndromic deafness cases. We have previously designed the conditions for linkage studies of this highly heterogeneous disorder [Guilford *et al.* (1994) *Nature Genet.* 6, 24–28]. Here, using a similar approach, we have studied the segregation of a gene responsible for congenital, profound and fully penetrant sensorineural deafness in a consanguineous family living in an isolated region of Lebanon. A maximum lod score of 8.03 (θ = 0.00) was detected with a new polymorphic marker, AFMa052yb5 (D2S2144). Observed recombinants and homozygosity mapping define a maximum interval of 2 cM for this gene, *DFNB6*, which lies between AFMb346ye5 (a new polymorphic marker) (D2S2303) and AFM254vc9 (D2S174) on chromosome 2p22-23.

INTRODUCTION

Deafness is the most predominant hereditary sensorineural disease in humans. One in 1000 newborn children is affected by deafness. Seventy percent of the cases of congenital deafness are nonsyndromic. The fraction of hereditary forms of nonsyndromic prelingual deafness is estimated to be ~60% in developed countries (2). This proportion is expected to be lower in developing countries due to the higher frequency of environmental causes, bacterial and viral infections or ototoxic drugs. However, in such countries, among the populations characterised by a high level of consanguinity, the contribution of hereditary forms is expected to be higher, since the autosomal recessive mode of inheritance is the most frequent [77% of the cases in UK (3)]. These recessive forms are essentially monogenic sensorineural diseases and are estimated to involve several dozen genes. They are generally more severe than the autosomal dominant forms.

Up until 1994, none of the genes responsible for recessive nonsyndromic deafness (*DFNB*) had been localised on human chromosomes. The difficulty of mapping these genes is due to a combination of the following obstacles: the high genetic heterogeneity of deafness, the absence of clinical characteristics distinguishing between the various genetic forms of sensorineural deafness and the frequency of marriages between deaf individuals in developed countries (in the US, up to 90% of married deaf individuals are married to a deaf person). We have circumvented these difficulties by analysing large and highly consanguineous families affected by deafness and living in geographically isolated regions for several generations. This minimizes the risk of several deafness genes segregating within the same family. Among the deaf families, we have selected those in which segregation of the trait suggests transmission of a single disease gene. By studying families living in remote villages from Northern and Southern Tunisia, we have mapped the first two genes responsible for recessive sensorineural deafness, *DFNB1* and *DFNB2*, to 13q11 (1,4) and 11q13.5 (5) respectively. Three additional *DFNB* genes have since been localised, *DFNB3* to 17p-17q12 (6), *DFNB4* to 7q31 (7) and *DFNB5* to 14q (8).

Several lines of evidence indicate that the migration of populations across or around the Mediterranean sea have contributed to the dispersion of some mutated genes (9,10) and of some particular diseases, such as periodic disease (11). In order to document the mutated genes responsible for recessive sensorineural deafness around the Mediterranean sea, we have now extended our study to affected families living in Lebanon. In this country, 17 religious communities have been identified which correspond, to some extent, to different ethnic groups (12). The incidence of consanguineous marriages is variable from one group to another. In one of these, the Moslem Sunnite community, whose members are of Arab descent and settled in Lebanon in the VIIth century, the consanguinity is estimated to be 24% (13). Here, we report on the genetic analysis of a prelingual and fully penetrant deafness in a sunnite consanguineous family living in an isolated village of Northern Lebanon. We have, thereby, localised a new gene, *DFNB6*, which maps to chromosome 2p.

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RESULTS

Clinical features

All family members underwent a general and an otoscopic clinical examination (see Materials and Methods). In affected individuals, no associated symptom such as external ear abnormality, mental retardation, eye or renal or integumentary disorder was noted. For affected children, deafness was noticed by their parents either before they reached 2 years of age, in the cases of a first affected child, or at birth in affected families. None of these congenitally deaf children seemed to have balance problems, however no serious evaluation of this function was performed. No evidence for any acquired risk factor predisposing to hearing loss was noticed in affected individuals.

Audiometry showed no response at 100 dB for frequencies superior to 1000 Hz in all affected subjects and no response at 80 dB for frequencies up to 1000 Hz. In affected children, no auditory brainstem response (ABR) was observed up to 100 dB. In the parents, who are obligate carrier heterozygotes, audiometric tests were normal.

Linkage analysis

We first excluded the three loci already known to be responsible for recessive sensorineural deafness, *DFNB1* (1,4), *DFNB2* (5) and *DFNB3* (6). Locus *DFNB5* (8), for which the markers were made available to us prior to publication, could then be excluded. We also tested a putative linkage to loci previously identified as responsible for dominant sensorineural deafness, *DFNA1* (14), *DFNA2* (15), *DFNA4* (16) since we have shown that a single locus might be responsible for both dominant (*DFNA3*) and recessive (*DFNB1*) forms of deafness (17). Analysis of 160 polymorphic markers from the Généthon collection (18), spaced ~20 cM apart, allowed us to exclude ~70% of the genome. Significant linkage was found with marker AFM242yd8 (locus D2S171) on chromosome 2 which gave a maximum lodscore of 5.9 ($\theta = 0.03$). To investigate the effect of hypothetical incomplete penetrance, the analysis was repeated assuming a penetrance of 80%. A maximum lod score of 5.7 confirmed the evidence for linkage.

The adjacent loci were then tested: on the distal side, D2S305 (10 cM away), D2S310 (7 cM) and D2S144 (0 cM); on the proximal side, D2S170 (5 cM away), D2S365 (4 cM), D2S174 (3 cM) and D2S158 (3 cM). Analysis of the recombinants in generation V (individuals V1, V16 and V19) mapped the gene proximal to D2S310 (Fig. 1). In generation IV, recombinant individuals IV1, IV2 and IV3 and in generation III, recombinant individuals III7 and III8 narrowed down the localisation interval to D2S171–D2S174, a distance of <3 cM (see Fig. 1). In order to refine this mapping, two new polymorphic markers within this interval were tested, markers AFMb346ye5 (D2S2303) and AFMa052yb5 (D2S2144) (see Materials and Methods) which map 1 and 2 cM proximal to D2S171 respectively. The recombinant individual IV2 maps the gene proximal to D2S2303, thus defining an interval, D2S2303–D2S174, of <2 cM. A maximum lodscore of 8.03 ($\theta = 0.00$) was observed with D2S2144 (marker AFMa052yb5). Interestingly, all affected individuals are homozygous for this polymorphic marker located within the interval, thus confirming the mapping data.

DISCUSSION

The gene responsible for non-syndromic, sensorineural profound deafness in this Lebanese family represents, so far, the sixth *DFNB* gene mapped to human chromosomes. The present results map the *DFNB6* gene to 2p22-23, since the marker D2S165 located at the same position as D2S174 (proximal limit of the interval) has been assigned to these chromosomal bands by *in situ* hybridisation (19). While we can consider that the conditions for mapping human genes responsible for recessive deafness have now been determined, by studying large pedigrees of geographically or culturally isolated populations (1,5–8), the difficulties in cloning the human *DFNB* genes are far from being solved. Both the positional cloning and the candidate gene approach come up against obstacles. Positional cloning relies upon the definition of a small chromosomal interval containing the disease gene. However, because of the extreme genetic heterogeneity, sensorineural deafness refined mapping can only be based on individual families giving significant lodscore. Hence, we are presently searching for other large deaf Lebanese families of the same cultural origin. Homozygous linkage disequilibrium is potentially extremely powerful but can only be applied to populations in which very few *DFNB* genes are segregating. The candidate gene approach will considerably accelerate the identification of the disease genes in the near future thanks to the expressed sequence tag (EST) chromosomal map. However, contrary to what is expected for genes involved in syndromic deafness, some of the *DFNB* genes might only be expressed in the inner ear neuroepithelium. Consequently, the ESTs, which are presently derived essentially from brain cDNA libraries, might be of limited interest for the identification of *DFNB* genes. Similar to what is known for the genes responsible for retinitis pigmentosa (20–23), the DFNB candidate gene approach would benefit from the identification of genes specifically expressed in the inner ear. These difficulties explain why none of these *DFNB* genes have yet been cloned. Only we have proposed, based on our mapping data, that the myosin VIIA gene, which is responsible for Usher syndrome type 1B (a disease that combines sensorineural deafness and retinitis pigmentosa), could also be responsible for the nonsyndromic deafness DFNB2 (24).

On the other hand, some deaf mouse mutants have turned out to be animal models for human deafness. Cloning of these mouse genes and their subsequent use in identifying human deafness genes has already demonstrated the interest of such an approach, as illustrated by the cloning of Splotch mutations (25) for Waardenburg type I (26,27), Microphthalmia mutations (28) for Waardenburg type II (29) and finally Shaker-1 (30) for Usher syndrome type 1B (24) (see ref. 31 for a review). For *DFNB6*, the regions of the mouse genome homologous to 2p22-23 are the distal part of chromosome 17 and the centromeric region of chromosome 12. No deaf mouse mutant has been mapped to the former region, while the latter carries the *asp-1* locus which is involved in the sensitivity to audiogenic seizures (32,33). Even if the phenotype of this mutant mouse seems rather different from the DFNB6 phenotype, the possibility that the corresponding genes are orthologous cannot be ruled out. This will be tested by the comparison of the refined subregional physical maps in both species.

Several genes have presently been mapped to chromosome 2p22-23. We will first consider as candidate genes malate dehydrogenase 1 (MDH1), since it is involved in the mitochon-

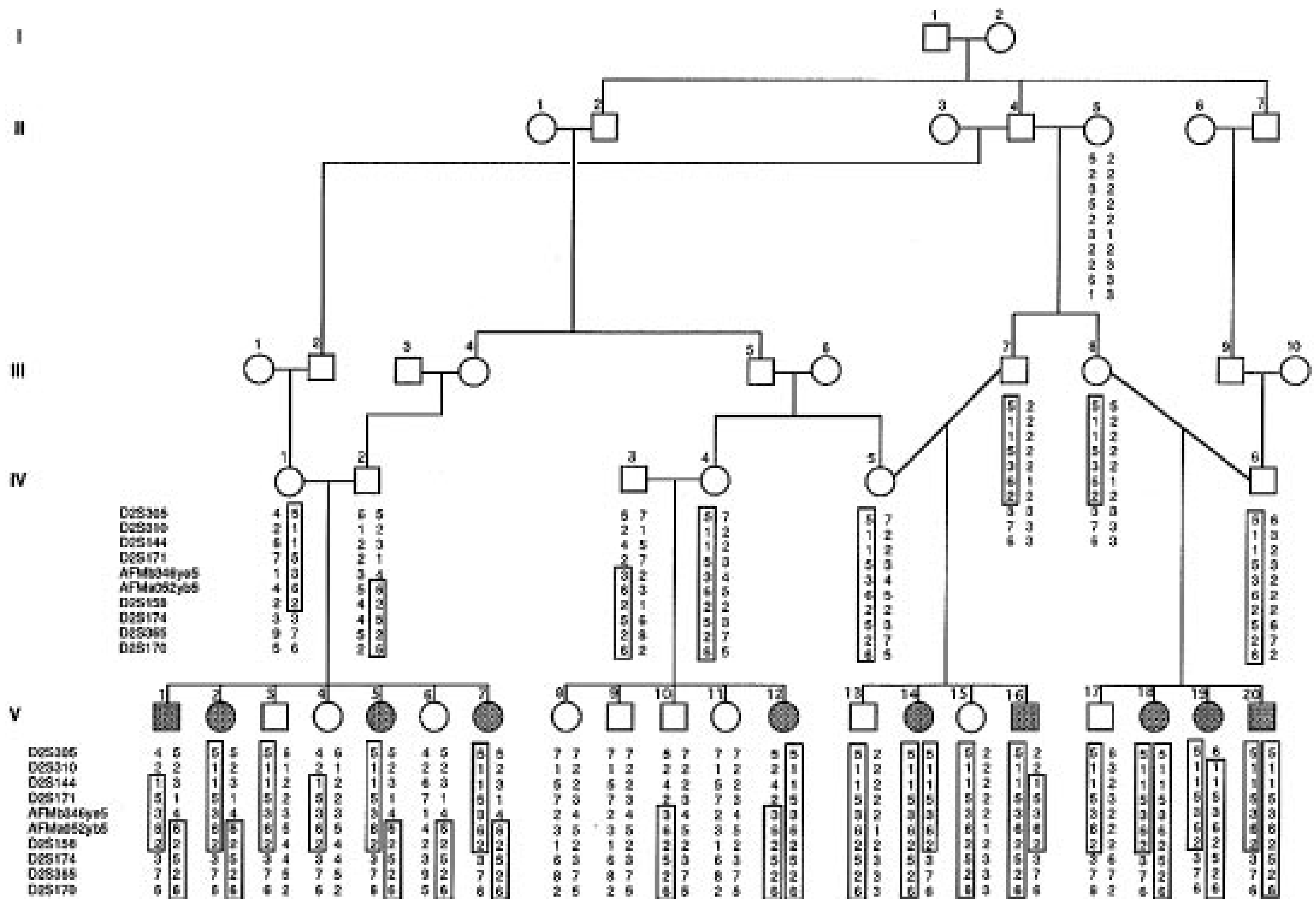

Figure 1. Family F, showing segregation of the linked loci. The haplotype associated with *DFNB6* in each individual is boxed. Dark symbols represent deaf individuals.

drial energetic pathway which is crucial in sensory hair cells (34) plus type III adenylate cyclase (ADCY3) (35,36) and calmodulin 3 (CALM3), since they may be involved in the auditory transduction pathway (37).

MATERIALS AND METHODS

Auditory tests

Informed consent was obtained from all 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 systemically performed (with Beltone 2000 clinical audiometer) as well as otoscopic examination for each individual over 5 years of age. For youngest children auditory brainstem responses (ABR) were tested using Lindar apparatus (RACIA).

Genotyping

DNA was extracted from 5 to 10 ml of blood by standard techniques. PCR reactions and hybridisations were carried out as previously described (1). The primer sequences of the Généthon markers, D2S144, D2S158, D2S170, D2S171, D2S174, D2S305, D2S310, D2S365 have previously been reported (18). The sequences of the primers for the new markers are as follows: AFMb346ye5 (D2S2303), 5′-TCCACCGTGGGCAACAAAG-3′ and 5′TGAGCTGCGTGACTGGGC-3′ and AFMa052yb5 (D2S2144), 5′-GCACTTGCTCACCGCT-3′ and 5′-TAGCCTT-CTGCCTCATACAT-3′.

Linkage analyses

Linkage analyses were carried out using the LINKAGE 5.1 program package (38). Two-point lod scores were calculated with the MLINK and ILINK programs and multi-point with LINKMAP. The deafness was assumed to be inherited in a recessive manner with complete penetrance. Recombination frequencies were assumed to be equal for both males and females. The allele frequencies of the polymorphic markers were assumed to be equal. One hundred fold change in the frequency of the linked allele resulted in only minor alterations (~10% variation) to the maximum two-point lod score. The frequency of the deafness gene was estimated to be 10^{-4}. Variation of this frequency between 10^{-3} and 10^{-5} resulted in negligible changes to the two-point lod scores. The 90% confidence limits were calculated using the '1 lod down' method (39).

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