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DFNB31, a recessive form of sensorineural hearing loss, maps to chromosome 9q32-34

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We report the identification of a novel locus responsible for an autosomal recessive form of hearing loss (DFNB) segregating in a Palestinian consanguineous family from Jordan. The affected individuals suffer from profound prelingual sensorineural hearing impairment. A genetic linkage with polymorphic markers surrounding D9S1776 was detected, thereby identifying a novel deafness locus, DFNB31. This locus could be assigned to a 9q32-34 region of 15 cM between markers D9S289 and D9S1881. The *whirler* (*wi*) mouse mutant, characterised by deafness and circling behaviour, maps to the corresponding region on the murine chromosome 4, thus suggesting that DFNB31 and *whirler* may result from orthologous gene defects. *European Journal of Human Genetics* (2002) 10, 210–212. DOI: 10.1038/sj/ejhg/5200780

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Introduction

Hearing impairment is the most frequent sensorineural defect in man. Approximately one in 1000 children is affected with prelingual deafness. In the majority of cases, deafness is isolated (or nonsyndromic), ie, hearing impairment is the sole symptom. Isolated deafness forms transmitted in the recessive mode are the most frequent (85%) and the most severe. Twenty-six different DFNB loci have been described to date (<http://www.uia.ac.be/dnalab/hhh/> and Petit *et al*¹ for review). Eleven of the corresponding genes have been identified (reviewed in Petit *et al*¹ and Verpy *et al*²). Using the approach based on homozygosity mapping in large affected consanguineous families, we have already identified five DFNB loci in the Lebanese population.^{3–7} Here, we report on the identification of a novel DFNB locus in a Palestinian family living in Jordan.

Methods

Informed consent was obtained from adult subjects and from parents of under-aged patients.

Auditory tests

Affected as well as unaffected members of the family underwent a general careful clinical and otoscopic examination. Pure tone audiometry with air and bone conduction at 250, 500, 1000, 2000, 4000 and 8000 Hz was performed (with a Beltone 2000 clinical audiometer) in every individual over 5 years of age. Air conduction pure-tone average (ACPTA) threshold in the conversational frequencies (0.5, 1 and 2 kHz) was calculated for each ear, and the hearing loss was classified as mild (20 dB ≤ ACPTA ≤ 40 dB), moderate (40 dB < ACPTA ≤ 70 dB), severe (70 dB < ACPTA ≤ 90 dB), or profound (ACPTA ≥ 90 dB). In younger children, the auditory function was explored by recording the audiometric brain-stem response (ABR). The patients' medical history was obtained, and earlier audiograms analysed.

Genotyping

DNA was prepared from 5–10 ml of blood. Fluorescent microsatellite markers (sequences published in Dib *et al*⁸)

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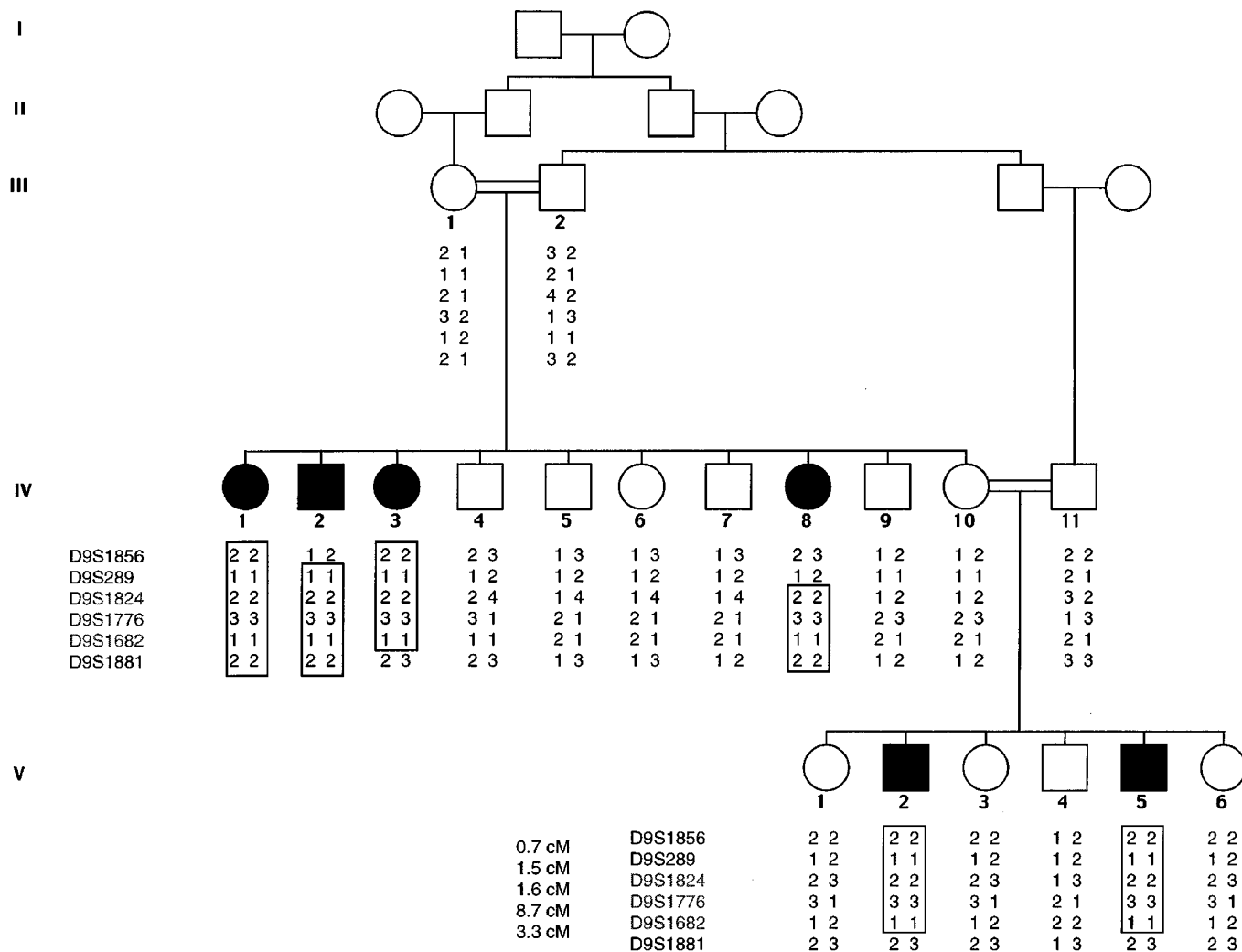


Figure 1 Segregation of 6 polymorphic markers from the 9q32-34 chromosomal region in family JO-CH. The genetic distance (in cM) between these markers⁸ is indicated at the bottom left. Horizontal double bars indicate consanguineous unions. Dark symbols represent deaf individuals. Analysis of the haplotypes in generation IV defines the DFNB31 minimal interval as the 15 cM region between D9S289 and D9S1881.

were used.⁹ The genome-wide screening was performed using 400 microsatellite markers that are spaced 10–12 cM apart (ABI PRISM[®] Linkage Mapping Set 2, Applied Biosystems). Multiplex PCR were performed with 4 markers. PCR products were analysed following electrophoresis on a 6% polyacrylamide gel.

Linkage analysis

LOD scores were calculated using the MAPMAKER/HOMOZ program¹⁰ (version 0.9), a computer package based on an algorithm especially designed for homozygosity mapping. The defect was assumed to be inherited on a recessive mode and fully penetrant. The disease allele frequency was estimated at 10^{-3} ; changing the disease allele frequency to

10^{-2} only slightly modified the lodscore value. The allele frequencies of the polymorphic markers and the meiotic recombination frequencies for males and females were assumed to be equal.

Results and Discussion

Clinical description

In family JO-CH, four individuals (IV-1, IV-2, IV-3 and IV-8) born from a marriage between two first cousins (III-1 and III-2), were affected by hearing loss (Figure 1). A second consanguineous marriage between first cousins (IV-10 and IV-11) resulted in six children among whom two had been recognised as deaf in the prelingual period. In the six affected

children IV-1, IV-2, IV-3, IV-8, V-2 and V-5 aged 32, 29, 27, 13, 11 and 5 years respectively, hearing impairment was bilateral and profound. No air-bone gap was observed in the audiograms, indicating that deafness is sensorineural. No other clinical sign was associated to deafness. Audiometric tests were normal in both parents. It can thus be concluded that a recessive form of isolated hearing loss is segregating in this family.

Linkage mapping

We undertook a screening of the whole genome using polymorphic markers spaced 10–12 cM apart.⁸ A linkage was detected only with locus D9S1776 on chromosome 9, thereby defining a novel deafness locus, DFNB31. Five other polymorphic markers from this chromosomal region were tested (see Figure 1). Two-point lodscores (Z_{\max}) at a recombination fraction $\theta=0.00$ were 5.20, 4.98 and 3.56 for D9S1824, D9S1776 and D9S1682, respectively. The calculated multipoint lodscore was 6.14. The homozygous region common to the six affected individuals extends between D9S1824 and D9S1682. The closest flanking markers limiting the interval are D9S289 and D9S1881, located at q32 and q34, respectively; these markers are spaced about 15 cM.⁸

Several DFNB forms have been shown to be allelic to dominant forms of isolated deafness (DFNA) and/or to syndromic forms of deafness (see Petit *et al*¹ and Petit¹¹ for reviews). So far, no DFNA form or syndromic hearing loss has been assigned to the DFNB31 chromosomal region.

Among the numerous deaf mouse mutants which have been reported, ten have already been proven to involve genes orthologous to those implicated in human isolated hearing loss (see^{1,12,13} for reviews). The region syntenic to the DFNB31 interval is located on the murine chromosome 4, 31 cM from the centromere. It contains the locus of the recessive mutant *whirler* (*wi*),^{14,15} which is characterised by profound deafness and vestibular dysfunction. Therefore, it is reasonable to propose that DFNB31 and *whirler* may represent defects of the same gene.

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