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# A novel locus for non-syndromic sensorineural deafness (*DFN6*) maps to chromosome Xp22

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**Non-syndromic X-linked deafness is highly heterogeneous. At least five different clinical forms have been described, but only two loci have been mapped. Here we report a Spanish family affected by a previously undescribed X-linked form of hearing impairment. Deafness is non-syndromic, sensorineural, and progressive. In affected males, the auditory impairment is first detected at school age, affecting mainly the high frequencies. Later it evolves to become severe to profound, involving all frequencies for adulthood. Carrier females manifest a moderate hearing impairment in the high frequencies, with the onset delayed to the fourth decade of life. Deafness was assumed to be X-linked dominant, with incomplete penetrance and variable expressivity in carrier females. The family was genotyped for a set of microsatellite markers evenly spaced at intervals of about 10 cM. We found evidence of linkage to markers in the Xp22 region (maximum lod score of 5.30 at $\theta = 0.000$ for *DXS8036* and for *DXS8022*). The position of the novel deafness locus (*DFN6*) was refined by haplotype analysis. Mapping of the breakpoints in two critical recombinants allowed us to define an interval for *DFN6*, delimited by *DXS7108* on the distal side and by *DXS7105* on the proximal side, and spanning a genetic distance of about 15 cM.**

## INTRODUCTION

Deafness is a very common disorder in humans. Genetic causes are responsible for about half of cases, with an incidence of approximately 1 in 1000 births (1). Deafness can be one among several clinical signs that define a distinct syndrome, but in most cases (70%) it is the sole clinical feature (2).

Non-syndromic deafness is highly heterogeneous, and estimates of the number of genes involved in this disorder range from about 30 to over 100 (3). X-linked transmission accounts for 1–5% of non-syndromic deafness (1). Several X-linked forms of isolated bilateral hearing impairment have been reported, and their classification is based on their audiological characteristics and age of onset (4,5).

Early-onset progressive sensorineural deafness (MIM 304700) was originally reported in a large Norwegian family (6). The locus, *DFN1*, was mapped to Xq22. However, upon careful re-study of the family, deafness was shown to be associated with blindness, dystonia, fractures and mental deficiency, and therefore it was reclassified as syndromic (Mohr-Tranebjaerg syndrome) (7).

Congenital sensorineural deafness type 1 (MIM 304500) includes several families in which only males are affected (5). A locus was postulated (*DFN2*), but no mapping data are available. Type 2 (MIM 300030) was reported in one family in which carrier females manifested mild to moderate hearing loss of delayed onset. The locus, *DFN4*, was mapped to Xp21.2 (8).

The locus for progressive mixed deafness, with stapes fixation and perilymphatic gusher upon stapes surgery (MIM 304400), *DFN3*, was mapped to Xq21.1 (9–11). Mutations in the gene encoding the POU3F4 transcriptional regulator were shown to be responsible for deafness in patients from several *DFN3* families (12). However, in other *DFN3* families the genetic defect has not yet been found.

In high-frequency sensorineural deafness (MIM 304590) the hearing loss is non-progressive and limited to the 1500–8000 Hz range (13). The genetic locus remains unmapped.

In moderate sensorineural hearing loss (MIM 304600) onset occurs at about 10 years of age and slowly progresses, but not beyond a moderate impairment (14,15). There is no mapping information available.

Here we report a Spanish family affected by a previously undescribed X-linked form of isolated sensorineural deafness. After excluding linkage to known loci for isolated hearing loss on the X chromosome, this novel locus was mapped to Xp22 in a 15 cM interval.

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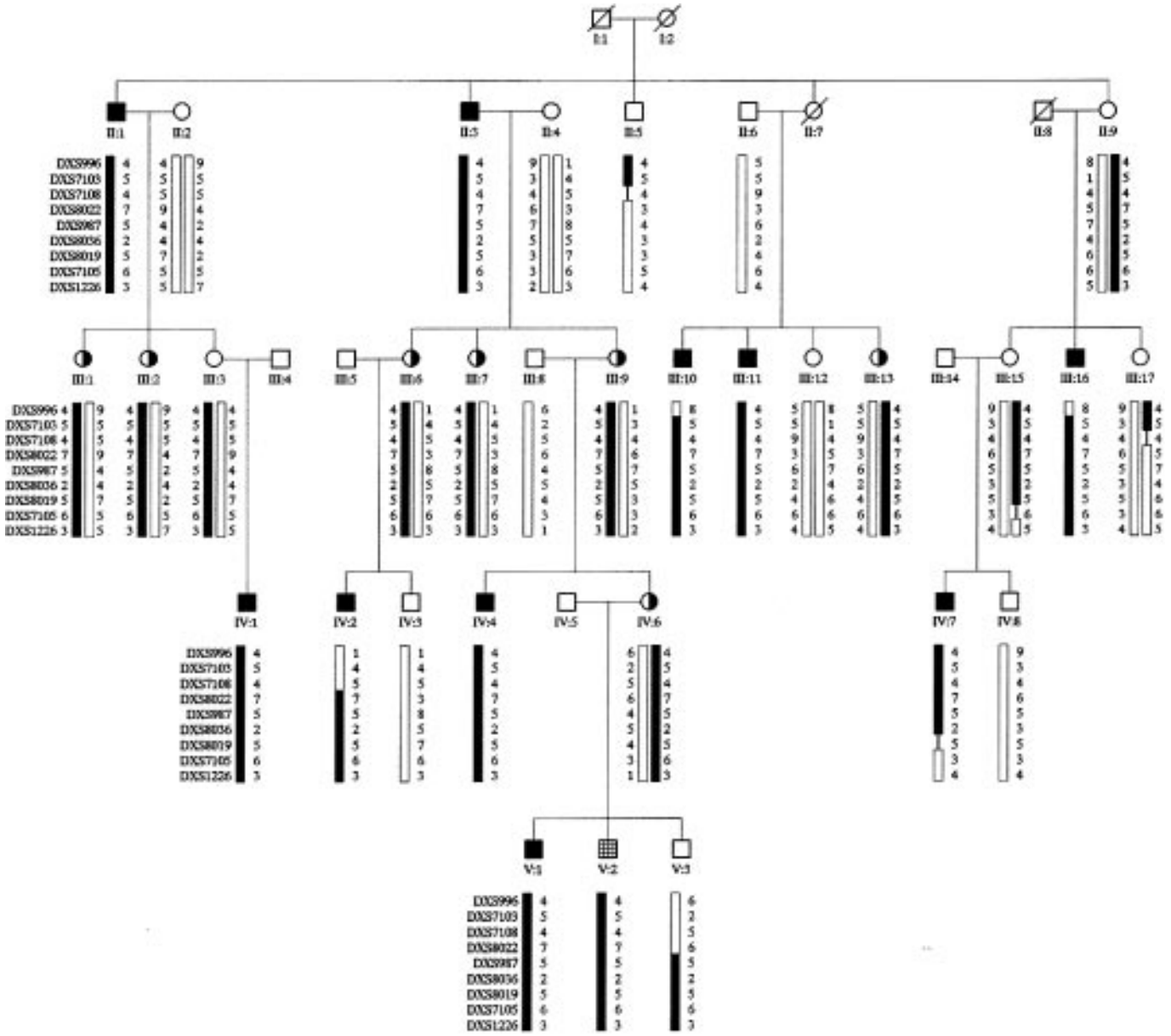

**Figure 1.** Pedigree and haplotype analysis of family S005. The complete family consists of 80 members, but only those relevant to the study are presented here. Haplotypes are represented by bars, with the haplotype associated to deafness in black, in order to show the recombinations. A thin line between two bar segments indicates that the marker was not informative for mapping the breakpoint. Black squares, affected males. Half-shaded circles, affected carrier females. Cross-hatched square, a 5 year old male with mild hearing loss just in the high frequencies.

## RESULTS

### Clinical features

We describe a five-generation family with X-linked non-syndromic deafness. Audiological assessment of 35 members of four generations revealed 10 affected males and seven affected females (Fig. 1). In this family, hearing loss is bilateral, sensorineural and progressive. Vestibular functions are normal and there are no complaints of tinnitus. In affected males, the auditory impairment is of postlingual onset. It is first detected at school age (5–7 years of age), affecting mainly the high frequencies in the audiogram (Fig. 2A). Later it evolves to become severe or profound, involving all frequencies for adulthood (Fig. 2B). Affected females manifest bilateral, moderate hearing impairment that affects the high frequencies (Fig. 2C). It appeared earliest in a 30-year-old carrier female, but for the other affected women the hearing loss was evident in the fourth decade of life.

### Linkage analysis

Deafness was assumed to be X-linked dominant, with incomplete penetrance and variable expressivity in carrier females. Seven out of 10 carrier females were affected, suggesting a penetrance of about 70%. Thirty-two members of the family were considered informative for linkage. Individual V-2 is only 5 years old, and

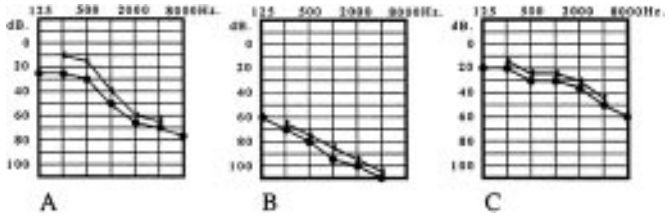


**Figure 2.** Audiograms of three affected members of family S005. Deafness is bilateral with a very similar pattern for both ears. For clarity-purposes, only results for the right ear are presented. Dots, air conduction. <, bone conduction. (**A**) A 9 year old male (individual V-1) at an early stage of the hearing loss, that involves mainly the high frequencies. (**B**) Individual III-11 illustrates a later stage. He is a 47 year old male whose hearing impairment is severe, affecting all the frequencies. (**C**) An affected 54 year old carrier female (III-9). Note the high-frequency hearing loss, resembling the early stages of affected males.

although his audiogram suggests a mild hearing loss just in the high frequencies, he was not included in the linkage analysis. His younger brother (individual V-3), only 2 years old, is below the age of onset and was also excluded from the study.

Firstly, we excluded linkage to the two non-syndromic deafness loci already mapped to chromosome X, *DFN3* and *DFN4*. Then, the family was genotyped for a set of microsatellite markers evenly spaced, at intervals of about 10 cM, along the X chromosome (16). We found evidence of linkage to marker *DXS8036* in the Xp22 region (maximum lod score of 5.30 at $\theta = 0.000$). Genotyping the family for additional markers of this region confirmed the linkage (Table 1).

### Haplotype analysis

The position of the novel deafness locus (*DFN6*) was narrowed down by haplotype analysis. Seven recombinants were identified (Fig. 1). Two of them define the limits for the interval that must contain the *DFN6* locus. The affected individual IV-2 has a recombination between *DXS7108* and *DXS8022* that locates *DFN6* centromeric to marker *DXS7108*. In individual IV-7, also affected, the recombination took place between *DXS7105* and *DXS8036*, and maps *DNF6* telomeric to marker *DXS7105*. Therefore, the critical interval for *DFN6* is delimited by *DXS7108* on the distal side, and by *DXS7105* on the proximal side, and it spans a genetic distance of about 15 cM.

## DISCUSSION

At least five different clinical variants of X-linked non-syndromic hearing loss have been reported (4,5). Here we describe a novel clinical form that differs from those previously known in audiological features, age of onset, progression and severity. Firstly, it is purely sensorineural, without any conductive component, and so it differs from most of the *DFN3* cases (MIM 304400), the majority of which show a mixed type of deafness. Secondly, it is not congenital like the *DFN2* (MIM 304500) and *DFN4* (MIM 300030) variants, since onset occurs at about 5–7 years of age. Thirdly, although the audiograms of patients in the early stages may resemble those reported by Wellesley and Goldblatt (13) (high-frequency sensorineural deafness, MIM 304590), the latter is non-progressive, whereas the variant described here is progressive, evolving to affect all frequencies in adulthood. Finally, it is severe to profound, which contrasts with the moderate sensorineural hearing loss reported in MIM 304600 (14,15).

In spite of the profusion of X-linked clinical forms, up to now only two genetic loci for non-syndromic deafness had been mapped on the X chromosome: *DFN3*, on Xq21.1, and *DFN4*, on Xp21.2. In the family described in this work we have excluded both loci, and found linkage of the novel deafness locus (*DFN6*) to markers in the Xp22 region. Two key recombinants (individuals IV-2 and IV-7) were detected in the haplotype analysis, which set up the limits of a 15 cM critical interval for *DFN6*, between markers *DXS7108* and *DXS7105*. Other microsatellite markers in this interval (*DXS7104*, *DXS1224*, *DXS7101*, *DXS7163*, *DXS999*) were not informative for further refinement of the location of the breakpoints. It should be noticed that there is another key recombinant in the family, individual V-3. He has a recombination between markers *DXS8022* and *DXS987*, that divides the critical interval in a distal part of 7 cM, and a proximal part of 8 cM. However, this individual is only 2 years old and his clinical status cannot be ascertained since he is under the age of onset of the hearing loss. In the absence of additional families with this X-linked form of non-syndromic deafness, careful attention to the clinical evolution of this individual should be paid in the future.

**Table 1.** Two-point lod scores between Xp22 marker loci and the *DFN6* gene

| | Recombination fractions (θ) | | | | | | | $Z_{max}$ | $\theta_{max}$ |
|---|---|---|---|---|---|---|---|---|---|
| Marker | 0.00 | 0.01 | 0.05 | 0.10 | 0.20 | 0.30 | 0.40 | | |
| *DXS996* | –∞ | –3.14 | –1.11 | –0.32 | 0.28 | 0.40 | 0.29 | 0.40 | 0.297 |
| *DXS7103* | –∞ | 1.02 | 1.59 | 1.69 | 1.52 | 1.13 | 0.61 | 1.69 | 0.102 |
| *DXS7108* | –∞ | –0.86 | –0.19 | 0.06 | 0.22 | 0.22 | 0.14 | 0.24 | 0.245 |
| *DXS8022* | 5.30 | 5.22 | 4.89 | 4.45 | 3.50 | 2.42 | 1.22 | 5.30 | 0.000 |
| *DXS987* | 4.70 | 4.63 | 4.33 | 3.94 | 3.09 | 2.13 | 1.06 | 4.70 | 0.000 |
| *DXS8036* | 5.30 | 5.22 | 4.89 | 4.45 | 3.50 | 2.42 | 1.22 | 5.30 | 0.000 |
| *DXS8019* | 4.70 | 4.63 | 4.33 | 3.94 | 3.09 | 2.13 | 1.06 | 4.70 | 0.000 |
| *DXS7105* | –∞ | 0.92 | 1.44 | 1.50 | 1.29 | 0.90 | 0.42 | 1.50 | 0.090 |
| *DXS1226* | –∞ | 0.34 | 1.49 | 1.78 | 1.68 | 1.25 | 0.63 | 1.80 | 0.126 |

Among the genes already mapped to the above mentioned interval, no obvious candidates to be responsible for *DFN6*-type deafness are found (17,18). In addition, no mutation causing hearing impairment in mice mutants has been mapped to the homologous region in the mouse X chromosome (19,20). Therefore, progress in identifying the *DFN6* gene will depend on the refinement of its genetic and physical location, as well as on the isolation of candidate cDNAs from the critical interval.

The genes for numerous syndromes involving hearing loss have been mapped to the X chromosome (5). The possibility remains that some of them are allelic to some forms of non-syndromic deafness, the clinical differences being due to different mutations in the same gene. It is also possible that the genetic defect in a syndrome involves two or more contiguous genes. These hypotheses have been considered in Usher syndrome type 1 B (progressive retinitis pigmentosa plus congenital hearing loss) and a non-syndromic autosomal recessive deafness. The loci responsible for these disorders, *USH1B* and *DFNB2*, respectively, map to the same region, 11q13.5 (21,22). A similar situation has been found in Pendred syndrome (congenital deafness plus thyroid goitre) and another non-syndromic autosomal recessive deafness (*DFNB4*). Both loci map to the same region in 7q31 (23,24). With regard to Xp22, two loci responsible for syndromes that include deafness have been mapped: *OASD*, the locus for X-linked recessive ocular albinism and deafness (MIM 300650), and *CLS*, the locus for the Coffin-Lowry syndrome (MIM 303600).

The association of X-linked ocular albinism and sensorineural deafness was described in a large Afrikaner family with seven affected males (25). Patients manifested high-frequency hearing loss in their fourth or fifth decades of life, and later the progression was slow. Audiograms of carrier females were normal. The *OASD* locus was mapped to Xp22.3, in the same region as *OA1*, the locus for isolated X-linked ocular albinism (26). It has been suggested that *OA1* and *OASD* may be allelic (26). If this were true, *OASD*/*OA1* and *DFN6* would be different entities, since *OA1* is distal to *DXS7108* (17,18) whereas *DFN6* is proximal to this marker. The comparison between clinical features of the hearing loss in *OASD* and in *DFN6* reveals differences in age of onset and progression, and in clinical status of carrier females. However, the hypothesis that *OASD* may be due to a deletion involving both *OA1* and *DFN6* cannot be formally ruled out.

Coffin-Lowry syndrome (CLS) is characterized by mental retardation and skeletal anomalies (5). In some pedigrees it is also associated with sensorineural deafness (27) that may be early-onset or congenital. Both males and carrier females are affected, in accordance with an X-linked dominant pattern of inheritance. The *CLS* locus has been mapped to a 3.4 cM interval in Xp22, between markers *DXS7163* (distal limit) and *DXS365* (proximal limit) (28). This interval is fully included in the *DFN6* interval, close to its proximal limit. We find it unlikely that *CLS* and *DFN6* may be allelic, but again the hypothesis of contiguous gene defects cannot be excluded.

The clinical diversity of X-linked non-syndromic hearing loss probably corresponds with a comparable genetic heterogeneity. The work reported here adds a novel clinical variant and a novel locus to the picture. Mapping of the genes involved in the clinical forms already reported should help to establish a classification useful for both the clinician and the geneticist.

## MATERIALS AND METHODS

### Family data

Clinical examination was performed on all patients and on their living relatives that were considered informative for linkage analysis. Pure-tone audiometry was carried out with a Maico MA41 audiometer. Frequencies tested for air conduction were 250, 500, 1000, 2000, 4000, and 8000 Hz. Frequencies tested for bone conduction were 250, 500, 1000, 2000, and 4000 Hz. Otoscopic examination, tympanometry with acoustic reflex testing, and tuning fork tests were carried out systematically to rule out a conductive hearing loss. For all patients, the hypothetical influence of environmental factors such as exposure to noise, otoacoustic trauma, treatment with ototoxic drugs, or ear infections, was excluded. No syndromic features were revealed by ophthalmologic, tegumentary, and renal exploration.

### Genotyping

DNA was extracted from peripheral blood by the salting out method (29). Data concerning the microsatellite markers and primers that were used in this study have been previously published (16). PCR was performed in a total volume of 15 µl in a Perkin-Elmer 9600 DNA amplifier, using standard conditions (16). The amplification program consisted of three steps: an initial cycle composed of denaturation at 94°C for 2 min, annealing at 58°C for 1 min, and extension at 72°C for 1 min; 30 cycles, each one composed of denaturation at 94°C for 40 s, and annealing/extension at 56°C for 30 s; a final cycle of extension at 72°C for 2 min. Alleles were resolved on 5–6.5% non-denaturing polyacrylamide gels and revealed by ethidium bromide staining.

### Linkage analysis

Linkage analysis was carried out using the LINKAGE 5.1 software package (30). Deafness was coded as an X-linked dominant disorder with incomplete penetrance (70%) in carrier females. Its gene frequency was set to 0.00001. Allele frequencies were estimated from at least 30 chromosomes of unrelated individuals. Two-point lod scores were calculated with the MLINK and ILINK programs. Extensive alterations of the penetrance value, the disease gene frequency, or the allele frequencies of microsatellite markers did not change the conclusions of the analysis.

Pedigree drawing and haplotype analysis were performed using the CYRILLIC 2 program (Cherwell Scientific Publishing Ltd.).

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