RESEARCH ARTICLE

Refinement of the locus for non-syndromic sensorineural deafness (DFN2)

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Abstract

Non-syndromic X-linked deafness is a rare form of genetic deafness in humans accounting for a small proportion of all hereditary hearing loss. Different clinical forms of non-syndromic X-linked deafness have been described, and most of these have been mapped. Here, we report a Chinese family affected by a congenital profound sensorineural hearing loss. All phenotypes of this family are clinically compatible with non-syndromic sensorineural deafness (DFN2). A maximum two-point Lod score of 2.32 was obtained at marker *DXS6797* ($q = 0.00$). Recombinants define a region of 4.3 cm flanked by markers *DXS6799* and *GATA172D05*. This region overlaps the previously reported DFN2 region by 2.0 cm.

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Introduction

Deafness is one of the most common sensory defects in humans, with an incidence of approximately 1 in 1000 births, and genetic causes are responsible for about half the cases of deafness (Cohen and Gorlin 1995). Deafness can be associated with other clinical features as part of a distinct syndrome, but in most cases (70%) it is the sole clinical feature (Bergstrom *et al*. 1971). Several X-linked forms of isolated bilateral hearing impairment have been reported and their classification is based on the mode of onset and audiogram data (Gorlin 1995). Genetically, the locus DFN1 had been assigned to the phenotype of progressive sensorineural hearing loss (OMIM304700). However, a further study of the same family revealed that deafness was associated with blindness, dystonia, fractures and mental deficiency, and therefore it was reclassified as a new syndrome, Mohr-Tranebjaerg syndrome (Tranebjaerg *et al*. 1995). Other studies have demonstrated that termination mutations of *TIMM8A* (*DDP*) gene are responsible for DFN1 (Jin *et al*. 1996). The DFN2 locus has been assigned to profound congenital sensorineural deafness, and mapped to Xq21 (Tyson *et al*. 1996; Manolis *et al*. 1999), whereas the locus DFN3, assigned to progressive mixed deafness with perilymphatic gusher, was mapped to Xq21.1 (Brunner *et al*. 1988; Wallis *et al*. 1988; Reardon *et al*. 1991), and it is caused by mutations in the *POU3F4* gene (Bitner-Glindzicz *et al*. 1995; de Kok *et al*. 1995). However, not all DFN3 families, which map to Xq13-q21, are found to show mutation in the *POU3F4* gene, and there may be another gene in this region affecting the same or similar phenotype (Dahl *et al*. 1995; de Kok *et al*. 1996). The locus for high tone sensorineural deafness, DFN4, was mapped to Xp21.2 (Lalwani *et al*. 1994). In this paper, we report data obtained from a

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Chinese family with progressive, sensorineural hearing loss, consistent with DFN2. After excluding two other deafness loci on the q arm of the X chromosome (DFN1 and DFN3), the DFN2 locus was located to a 4.3 cm region on X chromosome.

Materials and methods

Family data

All family members received careful examination, including pure-tone audiometry and electric evoked audiometry. For all affected individuals, the hypothetical influence of environmental factors such as exposure to noise, treatment with ototoxic drugs, or ear infections, was excluded. There was no evidence in this family, based on physical examination and history, that hearing impairment was part of some defined syndrome. After obtaining informed consent, peripheral blood was obtained from each family member. Genomic DNA was extracted using the QIAmp DNA Blood kit (Qiagen, Germany).

Genotyping

PCR amplification was performed in a total volume of 10 µl with a PTC-225 DNA Engine Tetrad (MJ Research, Inc., USA) using standard conditions. PCR products were electrophoresed on a LI-COR 4200L DNA sequencer (LI-COR Corp., USA) on 7% denaturing polyacrylamide gels with fluorescent-dye-labeled DNA markers. Data were collected and analysed with Base Image 4.1 and Gene Image 3.12 software (LI-COR Corp., USA). Linkage ready pedigree files were prepared using Gene Image 3.12 software.

Linkage analysis

We conducted two-point linkage analyses using the LINKAGE v.5.10 software package (Lathrop *et al*. 1984). In the linkage analysis, we modelled the disease as having a pattern of X-linked dominant inheritance, with complete penetrance in males. We set the affected allele frequency as 0.0001 and the polymorphic marker frequency to be equally distributed. Pedigree drawing and haplotype construction were done using the Cyrillic v.2.0 software (Cyrillic Software, UK).

Results

We typed every available individual of this family, using polymorphic markers on the X chromosome that were selected from among those listed on the Marshfield map (http://www.marshfieldclinic.org/research/genetics/Map_Markers/maps/IndexMapFrames.html). Two-point linkage analysis of the family showed a maximum Lod score of 2.32 with markers *DXS6797* and *DXS8100* at a recombination frequency of $q = 0.00$. The analysis of additional markers (*DXS6789 DXS6799 DXS8020 DXS1230 GATA-172D05 GATA165B12*, for data see table 1) allowed us to narrow down the candidate region as lying between markers *DXS6799* and *GATA172D05*. Pedigree and haplotype analyses were carried out, and three recombinants were identified (figure 1). The affected individual (III : 9) had a recombination between *DXS8020* and *DXS6779*. In another individual (III : 2), also affected, the recombination took place between *DXS8100* and *GATA172D05*. Combining this information indicates that DFN2 maps to a 4.3 cm region between the markers *DXS6799* and *GATA172D05*.

Discussion

Despite the profusion of X-linked clinical features, only three loci for non-syndromic deafness (DFN1, DFN2 and DFN3) have so far been mapped on to the q arm of the human X chromosome. The *TIMM8A* (*DDP*) gene has been found to be the cause of DFN1. Mutations in the *DDP* gene create early onset progressive sensorineural hearing loss, associated with syndromic conditions including visual impairment, dystonia, mental retardation and fragile bones. Linkage analysis also locates the *DDP* gene within

Table 1. Two-point Lod score result between the DFN2 locus and chromosome X markers.

	Two-point Lod score at *q*						
Marker	0.00	0.01	0.05	0.1	0.2	0.3	0.4
DXS6789	∞	0.28	0.83	0.93	0.81	0.54	0.22
DXS6799	∞	0.33	0.27	0.44	0.45	0.31	0.10
DXS8020	1.71	1.68	1.56	1.40	1.06	0.67	0.25
DXS1230	1.89	1.86	1.72	1.54	1.16	0.74	0.28
DXS6797	2.32	2.28	2.10	1.88	1.41	0.91	0.37
DXS8100	2.32	2.28	2.11	1.90	1.44	0.94	0.39
GATA172D05	∞	0.61	1.14	1.21	1.01	0.64	0.19
GATA165D12	∞	0.32	0.28	0.45	0.46	0.31	0.10

the DFN2 disease interval. To exclude the possibility of *DDP* gene being involved in the family studied here, we have screened the sequence of the *DDP* gene in two affected members of this family, and no mutations were found. We also sequenced *COL4A5*, a strong candidate gene in this DFN2 region (Kawai *et al*. 1996; Heidet *et al*. 1995), but found no mutations in the promoter and coding regions.

The audiological testing of all members in this family demonstrated that all affected males have a slow, steady, pure sensorineural hearing loss, and all of the obligate female carriers show a mild to moderate hearing loss (figure 2). Thus, the phenotype of this family is clinically consistent with DFN2. The data presented in this paper locate DFN2 in this Chinese family to an interval between *DXS6799* and *GATA172D05* on the X chromosome. Considering the previous studies in which DFN2 has been mapped to a locus between *DXS990* and *DXS1106*, a 6 cm segment (Tyson *et al*. 1996; Manolis *et al*. 1999), the overlapping defines the locus to an approximately 2 cm region between *DXS6799* to *DXS1106*. Finer mapping of DFN2 is an essential step towards identifying the responsible gene, yielding further information that may help to

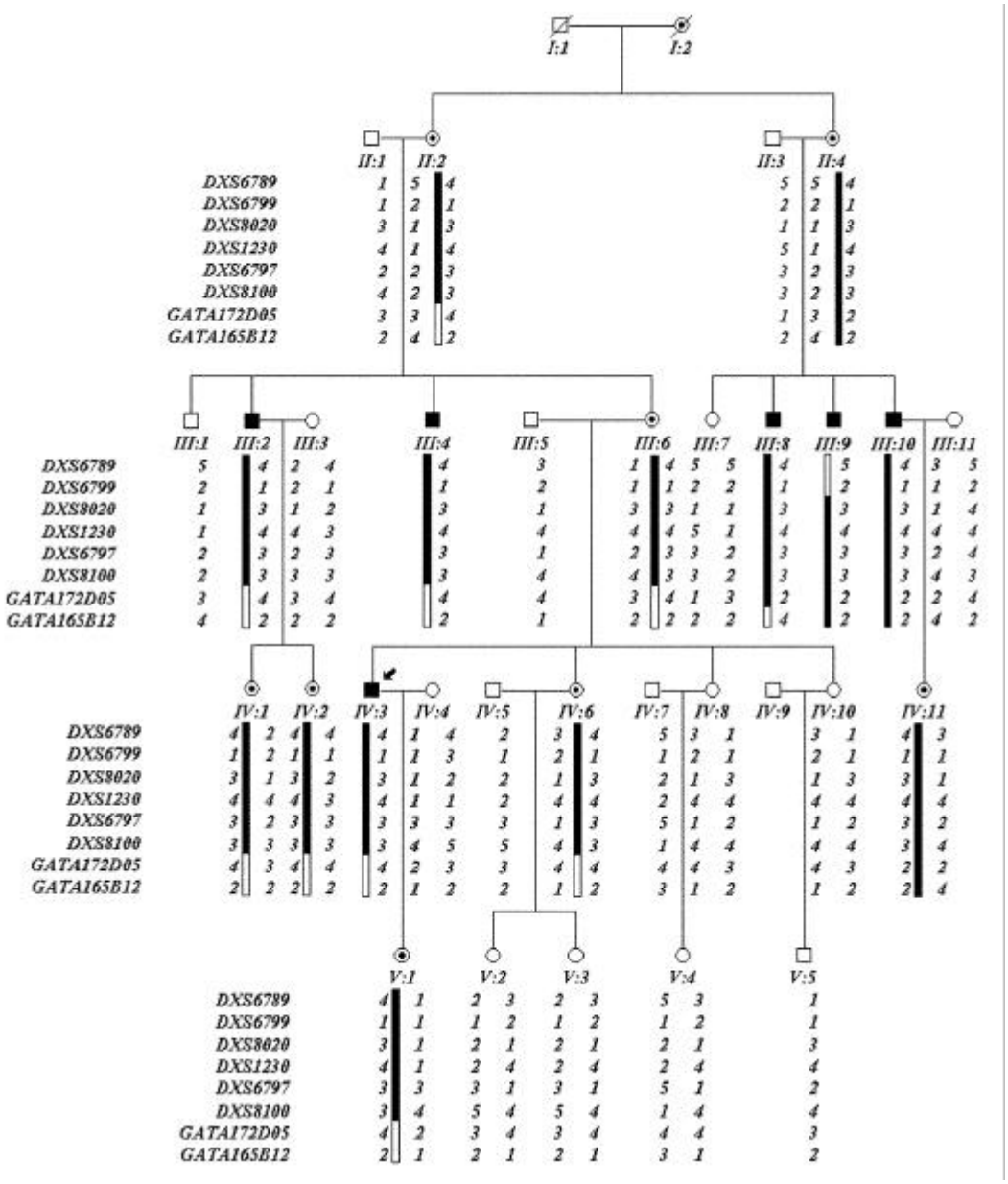

Figure 1. Haplotype analysis of X-linked deafness family. Haplotypes are shown for all available members with marker names at the left of each generation. Black bars represent disease-carrying haplotypes. Arrows indicate the initial probands.

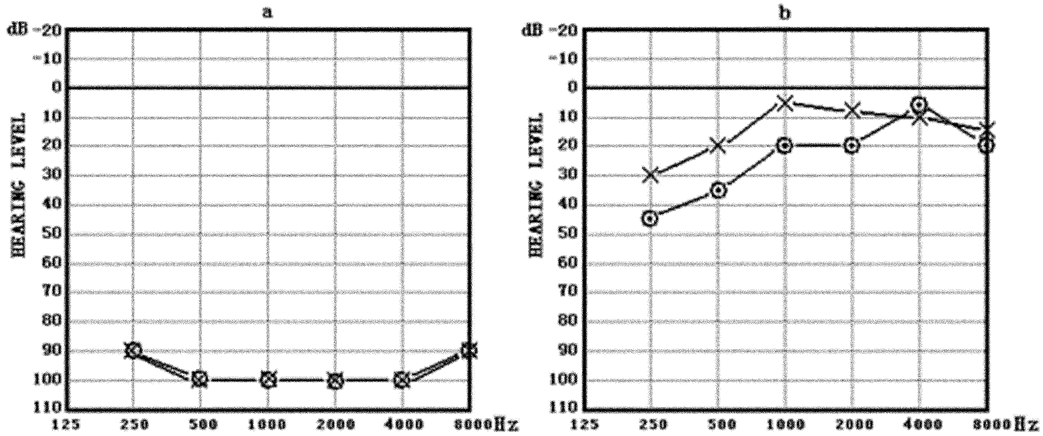

Figure 2. Audiograms of (a) an affected man, IV3, and (b) an obligate female carrier, IV6.

elucidate the pathology of this disorder. To date, no other genes mapped in this region appear to be a suitable candidate for this disease. A more complete expression map of the region, therefore, is likely to help in the task of isolating the DFN2 gene.

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