

## Letter to the Editor

# A novel autosomal dominant deafness locus (DFNA58) maps to 2p12-p21

### *To the Editor:*

Hearing loss (HL) is one of the most genetically heterogeneous disorders known. Post-lingual HL affects 10% of the population by the age of 60 years, with genetic causes playing an important role (1). Non-syndromic deafness represents 70% of all hereditary cases. Autosomal dominant inheritance probably accounts for 20% of all cases of non-syndromic hereditary deafness, usually with post-lingual onset and progressive manifestation (2). Although 41 loci associated with autosomal dominant non-syndromic sensorineural hearing loss (ADNSSL) have been mapped by linkage analysis in large families, to date, 22 different genes have been identified (3, 4) with practically no repetition, indicating the vast genetic heterogeneity involved and the potential of big deafness families to reveal new genes involving auditory system functions. In this study, we describe a Brazilian family with 12 individuals affected by bilateral post-lingual and progressive HL (Fig. 1).

The hearing impairment is initially presented as mild and affecting high frequencies in all patients (downsloping audiometric curve configuration) but becoming more severe and affecting all frequencies with time (flat audiometric curve configuration). Syndromic features were ruled out by physical examination and complete anamneses. No symptoms of vestibular dysfunction were reported. Age of onset varied from 18 to 45 years. The earliest documented onset was from patient VI:4 at the age of 18 years who also referred to episodes of bilateral tinnitus. She exhibited a mild hearing impairment (air conduction pure-tone average thresholds in the conversational frequencies of 0.5, 1, 2 and 4 kHz) with greater severity in high frequencies and showing a downsloping audiometric curve. Patient IV:8 referred to tinnitus and hearing impairment by the age of 27 years; by the age of 40 years, she presented moderate hearing impairment (downsloping audiogram), and at 51 years, a moderate hearing impairment in all frequencies

with a flatter audiometric configuration. Patient IV:3 showed a moderate HL in all frequencies at 51 years of age (flat audiometric curve). Patient V:9 noticed a hearing deficit by the age of 33 years and audiometry at 42 years indicated moderate bilateral HL. Patient IV:5 presented a bilateral severe hearing impairment with a downsloping audiogram at 26 years, which progressed to profound HL by 46 years with a flat audiometric curve. The penetrance rate was estimated to be 96% by 35 years and probably 100% by 45 years. Patient IV:11 had worked for several years exposed to extremely loud noises in the metallurgical industry, and his audiometry suggested noise-induced HL. Thus, individual IV:11 and all subjects younger than the age of 35 years who did not have documented hearing impairment (individuals indicated by ?) were coded as phenotypically unknown in the linkage analysis.

After obtaining appropriate informed consent from 32 family members, genomic DNA was extracted from peripheral blood by standard procedures using phenol/chloroform. LOD scores were calculated under an autosomal dominant mode of inheritance with a global penetrance of 96%, a disease allele frequency of 0.0001 and using marker allele frequencies according to estimates based on our own genotype data. Although this approach is less precise than applying an age-related penetrance to the analysis and scoring individuals as either affected or normal, the qualitative end result of the linkage analysis would probably be the same and not greatly reduce the estimated size of the detected locus unless we could identify other family members and involving new recombinations. Two-point LOD scores were calculated using MLINK from the FASTLINK package (5) and multipoint LOD scores using MERLIN (6). Initially, microsatellites close to the ADNSSL loci (DFNA1–DFNA39) were tested for linkage, and significantly negative two-point LOD scores were obtained, indicating involvement of a novel

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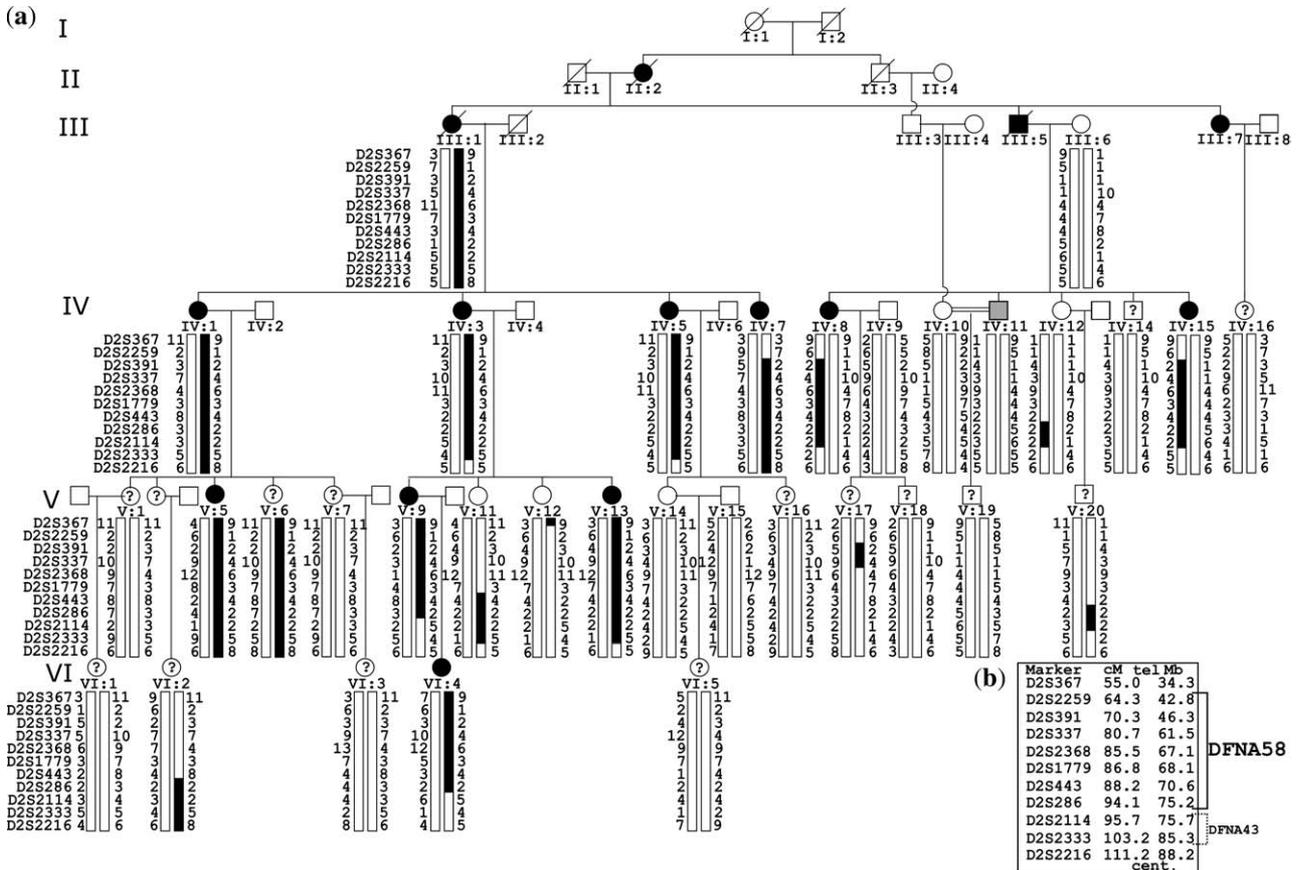


Fig. 1. (a) Pedigree showing the haplotypes of the 2p12-21 chromosomal region. The subjects younger than the age of 35 years who do not present hearing impairment are indicated by ?. Subject IV:11 is indicated in gray because he represents a phenocopy. (b) Map indicating the genetic (Marshfield map) and physical positions of the markers studied. We indicate that the DFNA43 locus was mapped between markers D2S2114 and D2S2333 and that the DFNA58 locus maps between markers D2S2259 and D2S2114.

locus in this family. Accordingly, a genome-wide scan was performed using 382 fluorescent-labeled microsatellites with an average spacing of 10 cM (ABI Prism Linkage Mapping Set 2.5; Applied Biosystems, Foster City, CA) and analyzed on MegaBACE 1000 automated DNA sequencer (Amersham Biosciences, Little Chalfont, UK). The genome-wide scan genotypic data of the 11 affected individuals and patient IV:11 (coded as phenotypically unknown) were analyzed to obtain multipoint LOD scores. Significantly positive LOD scores were only obtained with markers within the chromosomal region 2p12-p21, and additional markers and individuals were genotyped within this region. The highest two-point LOD scores of 3.47 ( $\Theta = 0$ ) and 3.35 ( $\Theta = 0$ ) were obtained with markers D2S391 and D2S337 in chromosomal region 2p13 (Table 1). Multipoint linkage analyses performed with 15 individuals, 11 affected individuals (IV:11 was not included because he was a phenocopy) and 4 documented normal-hearing individuals older

than the age of 35 years (III:6, IV:12, V:11 and V:12) yielded a peak LOD score of 4.14 (Fig. 2). This score is the theoretical maximum that could be obtained from this family using either MLINK or MERLIN. The interval for the novel deafness locus, DFNA58, was defined between markers D2S2259 and D2S2114 by analysis of the recombinant haplotypes in subjects IV:7, IV:8, IV:15 and VI:4 in combination with the multipoint LOD score distribution. Indeed, individual IV:11 whose clinical history indicated a noise-induced HL did not inherit the DFNA58 haplotype, and most probably, he is a phenocopy. The DFNA58 candidate chromosome region comprises 30 cM and, although it is close to DFNA43 (delimited by markers D2S2114 and D2S2333) (7), the two loci do not overlap.

The DFNA58 locus encompasses at least 154 known genes and 44 of them are known to be expressed in the human, mouse and/or rat cochlea (8, 9). All coding exons and exon-intron boundaries of nine candidate genes *ATP6V1B1*,

Table 1. Two-point LOD scores calculated for markers in the 2p12-p21 chromosome region using a penetrance estimate of 96%

Markers	Recombination fractions ( $\Theta$ )						
	0.0	0.01	0.05	0.1	0.2	0.3	0.4
D2S367	-2.12	0.62	1.35	1.55	1.45	1.08	0.56
D2S2259	-5.77	-0.85	0.32	0.66	0.81	0.71	0.44
D2S391	3.47	3.41	3.16	2.83	2.12	1.33	0.50
D2S337	3.35	3.30	3.07	2.77	2.18	1.38	0.58
D2S2368	2.67	2.60	2.32	1.95	1.16	0.33	-0.16
D2S1779	1.18	1.16	1.09	0.98	0.72	0.44	0.16
D2S443	2.39	2.43	2.45	2.34	1.92	1.35	0.66
D2S286	1.66	1.63	1.51	1.35	1.01	0.64	0.27
D2S2114	-5.95	-1.39	-0.41	-0.01	0.19	0.12	0.05
D2S2333	-10.70	-4.30	-2.08	-1.13	-0.32	0.00	0.09
D2S2216	-8.10	-4.00	-2.00	-1.17	-0.43	-0.10	0.02

*ATP6V1E2*, *OTX1*, *NRXN1*, *KCNK12*, *SPTBN1*, *CHAC2*, *PIGF* and *GPR75* were sequenced using a DYEnamic ET Dye Terminator Cycle Sequencing Kit (Amersham Biosciences) and analyzed with the MegaBACE 1000. The most obvious candidate gene was *ATP6V1B1* because it had already been associated with an autosomal recessive syndromic form of deafness (distal renal tubular acidosis with sensorineural deafness – MIM 192132). No mutations were found in any of them. Some normal-hearing and deaf subjects reported non-recurrent renal infections and/or kidney stones. Because these are common renal disorders in the general population and they do not segregate with deafness or the DFNA58 haplotype in this family, the *ATP6V1B1* gene is unlikely to be involved in the phenotype in this family.

In conclusion, we mapped DFNA58, a novel locus associated with autosomal dominant non-syndromic deafness, to chromosome region

2p12-p21, and no pathogenic mutations were found in nine candidate genes.

### Acknowledgements

The authors thank all family members for participation in this study and laboratory fellows for their collaboration, including Eliete Pardono, Ronaldo Serafim Abreu-Silva, Ana Carla Batissoco and Jihane Romanos for their invaluable help and Camila Juncansen, Katia Maria da Rocha and Martha Lima-Cozzo for technical support. We also thank Dr Diogo Meyer, Rodrigo Ramalho and Natale Cavaçana for helping with the LOD score calculations, Dr Edward Wilcox for sending us the primers to amplify markers close to DFNA loci and Dr Christian Kubisch for sending the primers to amplify *CHAC2*, *PIGF* and *GPR75* genes. This work was financially supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

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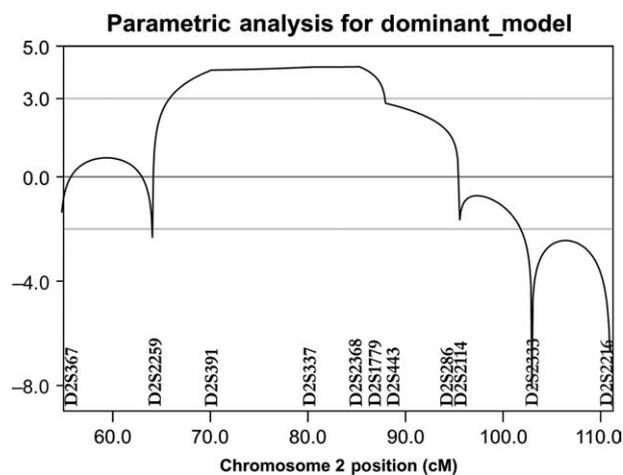


Fig. 2. Multipoint LOD scores calculated with MERLIN software for markers in the 2p12-p21 chromosome region using a penetrance estimate of 96%.

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