

LETTER TO JMG

DFNA49, a novel locus for autosomal dominant non-syndromic hearing loss, maps proximal to DFNA7/DFNM1 region on chromosome 1q21-q23

M A Moreno-Pelayo*, S Modamio-Høybjør*, A Mencía, I del Castillo, S Chardenoux, M Fernández-Burriel, M Lathrop, C Petit, F Moreno

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Approximately 1 in 1000 children is born with a serious permanent hearing impairment (pre-lingual deafness), and it is estimated that more than half of these cases in developed countries are due to genetic factors.^{1–3} The prevalence of hearing loss increases dramatically with age; it is estimated that approximately 5% of people under 45 years of age have a significant loss of hearing, increasing to approximately 50% by 80 years of age.¹ Age related late onset hearing loss (presbycusis) is a heterogeneous trait with many suspected causes.⁴ Genetic or environmental factors such as diabetes, mitochondrial mutations, or environmental noise exposure may contribute to the trait. In addition, hearing loss beginning at late childhood, youth, or later, clearly segregates as a monogenic autosomal dominant Mendelian trait in many families. The hearing loss phenotype in these families is usually non-syndromic—that is, the hearing loss is not associated with other anomalies, and it accounts for up to 20% of the cases of non-syndromic sensorineural inherited deafness. This type of hearing loss is usually progressive, affecting a particular range of frequencies in each case.³ To date, 36 loci for autosomal dominant non-syndromic sensorineural hearing loss (ADNSSL) have been mapped, and 17 deafness genes from these loci have been identified.⁶ These genes encode a wide variety of proteins; some have known function but for most, the underlying mechanisms leading to hearing impairment are uncertain. Given the few described mouse models for progressive hearing loss,⁷ our current understanding of post-lingual and progressive deafness relies on the identification of genes through human mapping studies. In this work we describe the mapping of a novel DFNA locus on chromosome 1q21–q23 segregating in a Spanish family with post-lingual and progressive hearing loss.

PATIENTS AND METHODS

Family data

We ascertained a four generation family (S277) segregating ADNSSL, through the Hospital Universitario Materno Infantil. The pedigree consists of 31 members, fourteen of them affected (fig 1). Informed consent was obtained from all study participants and from parents of subjects younger than 18 years. Clinical history interview and physical examination of members of this family ruled out environmental factors as the cause of the hearing loss. Peripheral blood samples were collected from 27 family members and DNA extraction was performed following standard methods. Tympanometry indicated proper functioning of the middle ear, and acoustic reflex thresholds showed the presence of recruitment in affected subjects. Clinical and instrumental evaluation did not reveal any evidence of syndromic features. Pure tone audiometry was performed to test for air conduction (125–8000 Hz) and bone conduction (250–4000 Hz).

Key points

- Autosomal dominant inheritance accounts for about 20% of the cases of hereditary non-syndromic sensorineural hearing loss (NSSL)—that is, hearing loss not associated with other clinical features. So far, 36 loci have been mapped in familial cases that segregate autosomal dominant NSSL (DFNA), and 17 genes have been identified.
- Here we report the location of a novel autosomal dominant deafness locus on 1q21–q23, DFNA49, found by studying a large Spanish family with non-syndromic, progressive mid-frequency hearing loss of post-lingual onset. A maximum lod score of 6.02 at $\theta=0$ was obtained for markers D1S3784 and D1S3785. Analysis of recombinant haplotypes placed the deafness locus within a 4 cM region defined by markers GDB:190880 and D1S3786.
- This genetic interval is proximal to and does not overlap with the previously identified loci, DFNA7 and DFNM1, on 1q21–q23.
- Screening of candidate genes within the DFNA49 interval (*KCNJ9*, *KCNJ10*, *ATP1A2* and *CASQ1*) did not reveal the mutation causing this deafness.

Where possible, results from previous audiological tests were collected.

Genotyping and linkage analysis

A wide genome scan was performed with 394 microsatellite markers distributed with an average spacing of 10 cM (ABI Prism Linkage Mapping Set 2; Applied Biosystems, Foster City, CA, USA). Markers for the exclusion of all known DFNA loci and for fine mapping of the critical interval were taken from the Généthon human linkage map⁸ and from the Marshfield chromosome 1 map (<http://research.marshfield-clinic.org/genetics>). Additional short tandem repeats (STRs) in the DFNA49 region were identified by inspection of publicly available sequence data (NCBI: <http://www.ncbi.nlm.nih.gov>). Flanking primers were designed for polymerase chain reaction (PCR) amplification of these

Abbreviations: ADNSSL, autosomal dominant non-syndromic sensorineural hearing loss; NSSL, non-syndromic sensorineural hearing loss; PCR, polymerase chain reaction; STRs, short tandem repeats

STRs. The amplicons were confirmed to be polymorphic microsatellite markers and deposited into the GDB database (<http://www.gdb.org>), being assigned the following D numbers: D1S3780 (GDB: 11511382), D1S3783 (GDB: 11511388), D1S3784 (GDB: 11511390), D1S3785 (GDB: 11511392), and D1S3786 (GDB: 11511394). The order of markers used in this work was established by integrating genetic and physical maps (NCBI).

Fluorescently labelled alleles were analysed in an ABI Prism 310 automated DNA sequencer (Applied Biosystems). Linkage analysis was performed using the Linkage 5.1 software package.⁹ Two point lod scores between the deafness locus and each marker were calculated under a fully penetrant autosomal dominant mode of inheritance, setting the disease allele frequency to 0.00001 and considering marker allele frequencies to be equal to each other.

DNA sequencing analysis

Four candidate genes, *KCNJ9* (MIM600932), *KCNJ10* (MIM602208), *ATP1A2* (MIM182340) and *CASQ1* (MIM114250) in the DFNA49 interval were screened by heteroduplex analysis and direct sequencing of PCR products generated from genomic DNA of affected subjects. Primers were designed to amplify each exon and adjacent intron-exon boundaries. PCR was performed by standard procedures as previously described.¹⁰ Heteroduplex analysis was carried out in mutation detection enhancement gels (BioWhittaker, Rockland, ME, USA) according to the manufacturer's protocol. Sequences of PCR products were analysed in an automated DNA sequencer (ABI Prism 310; Applied Biosystems).

RESULTS AND DISCUSSION

Clinical features

Affected subjects of the family present a symmetrical, bilateral, and progressive nonsyndromic sensorineural hearing loss. The hearing loss appears in the first decade of life in affected members. The earliest clinical evidence of hearing loss in the family was obtained from individual III:12 at the age of 8 years. Initially, the hearing loss in this family is moderate for low and mid frequencies and mild for high frequencies (4000–8000 Hz), so the affected members show in this stage a gently upsloping audiometric profile. Later, it progresses to moderate in the 125–250 Hz and 4000–8000 Hz ranges and to severe in the 500–2000 Hz range (U shaped audiometric profile) in the fourth decade. Linear regression analysis, based on all available audiograms from affected subjects, showed a 0.7 dB/year age linked progression of the hearing loss at all frequencies. Affected subjects of the family did not exhibit either tinnitus or clinical features suggestive of vestibular dysfunction.

Linkage analysis

Linkage to previously published loci responsible for autosomal dominant deafness (DFNA) was investigated in a core pedigree of 26 persons (subject IV:3 is 20 months old, clearly below the age of deafness onset, and therefore he was not included in the linkage analysis) obtaining negative results. A wide genome search was then performed to map the deafness locus, using a set of 394 microsatellite markers with an average spacing of 10 cM. Interestingly, the two point lod scores obtained for markers D1S498 (3.01 at theta = 0) and D1S484 (3.72 at theta = 0.05) on chromosome 1q21–q23

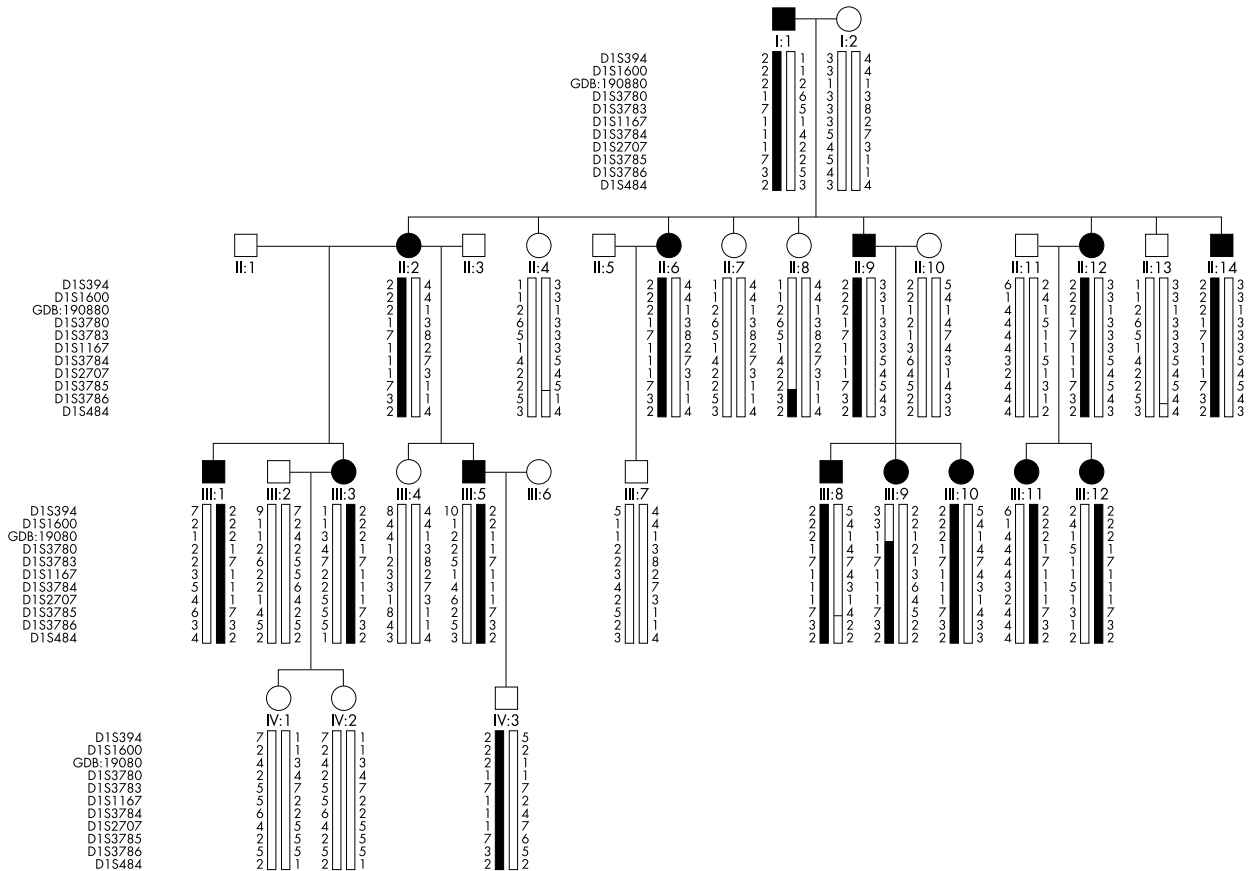


Figure 1 Pedigree and haplotype analysis of the Spanish family S277. Black symbols represent affected subjects. Haplotypes are represented by bars, with the haplotype associated with hearing loss in black. At the time of the study affected subjects III:12, III:11, III:10, III:9 and III:8 were 8, 14, 15, 17 and 18 years old respectively.

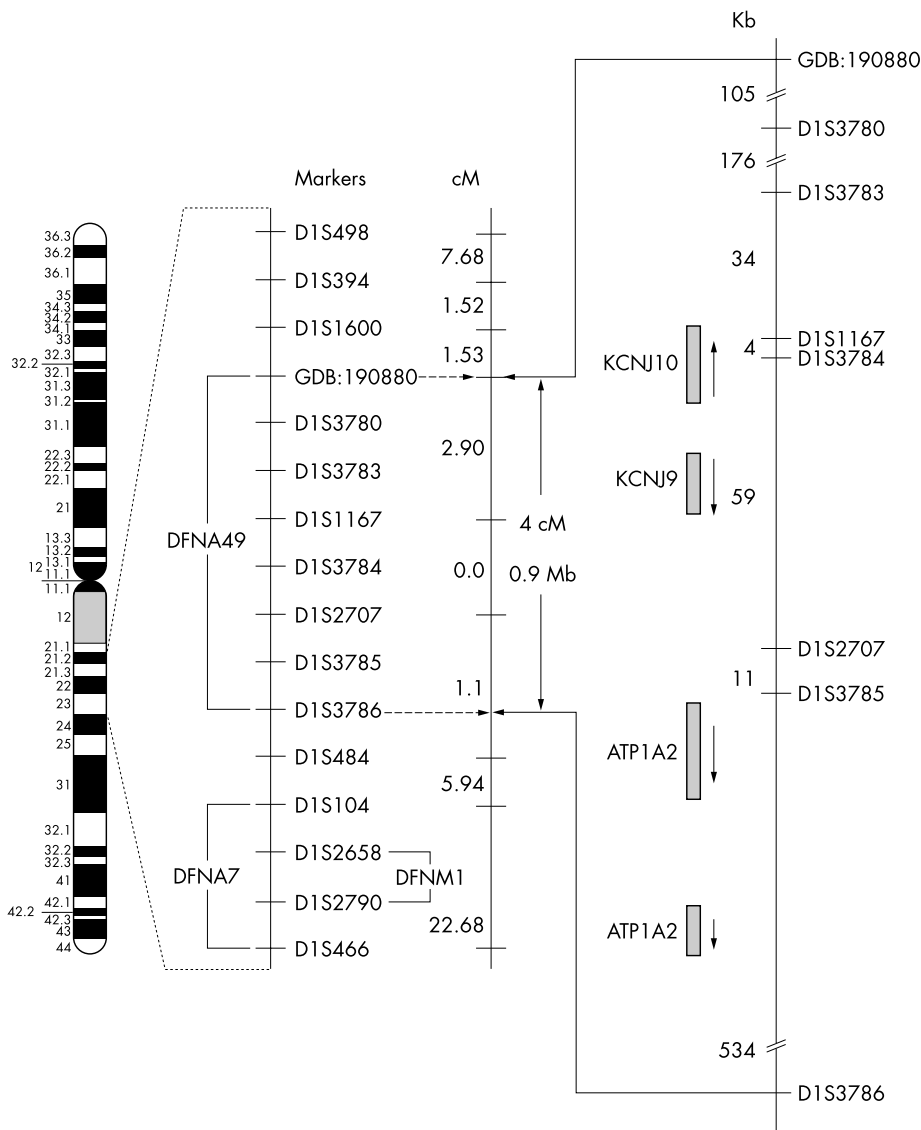


Figure 2 Physical and genetic maps of the chromosome 1q21–q23 region showing the localisation of DFNA49 and DFNA7/DFNM1 critical intervals. cM, centimorgan; kb, kilobase. *KCNJ9* (OMIM #600932); *KCNJ10* (OMIM #602208); *ATP1A2* (OMIM #182340) and *CASQ1* (OMIM #114250). For clarity, the genetic and physical distances are not represented to scale.

were suggestive of linkage to this region (fig 2). We then tested additional markers spanning this region, and evidence of linkage to markers D1S3780, D1S3783, D1S1167, and D1S2707 was found, with maximum two point lod scores of 6.02 at $\theta = 0$ for markers D1S3784 and D1S3785 (table 1). Extensive alterations of the disease gene frequency or the allele frequencies of microsatellite markers did not change the conclusions of the analysis. The inspection of recombinant haplotypes in subjects III:9 and II:8 placed the hearing loss locus between the proximal marker GDB: 190880 and the distal one D1S3786, which define a critical interval of about 4 cM for the novel locus, DFNA49 (fig 1 and 2). All the patients in the family share the same disease haplotype, which is also carried by the 20 month old child IV:3. Periodic audiometric examination of this subject will be performed to precisely determine when the hearing impairment begins in this family.

Another dominant locus for hearing loss (DFNA7) was mapped to a 22 cM region on 1q21–q23 between D1S104 and D1S466 in a Norwegian family¹¹ (fig 2). The DFNA7 interval has been reported to include the DFNM1 locus, a dominant modifier that suppresses the DFNB26 phenotype.¹² The

DFNA7/DFNM1 region had previously been excluded for linkage in our family. As shown in fig 2, a genetic distance of 6 cM separates this interval and the one defined for DFNA49. It should also be noted that the audiometric response associated with DFNA7 and DFNA49 in the reported families is clearly different. DFNA7 patients show a sharply sloping audiogram affecting the high frequencies, instead of the U shaped audiometric pattern found in the DFNA49 affected subjects.

Candidate gene analysis

No genes for syndromic deafness have been mapped to DFNA49 critical interval. This interval spans 0.9 Mb and includes 23 known genes and several predicted or poorly characterised genes according to the annotation in the NCBI database. Of these, we selected *ATP1A2*, *CASQ1*, *KCNJ10* and *KCNJ9* for screening of mutations (fig 2).

ATP1A2 consists of 23 exons¹³ and encodes the NaK-ATPase $\alpha 2$ subunit, which is responsible for the catalytic activity of the enzyme. In mouse cochlea, the $\alpha 2$ isoform is expressed in the spiral structures: ligament, limbus, and ganglion¹⁴, where it is believed to play an important role in the development

Table 1 Two point lod scores between 1q microsatellite markers and DFNA49

Marker	Recombination fractions (θ)							Z_{\max}	θ_{\max}
	0.00	0.01	0.05	0.10	0.20	0.30	0.40		
D1S394	−∞	3.93	4.27	4.11	3.38	2.40	1.20	4.27	0.05
D1S1600	−∞	3.34	3.72	3.59	2.98	2.11	1.04	3.72	0.05
GDB:190880	−∞	0.97	1.51	1.60	1.44	1.09	0.62	1.60	0.10
D1S3780	5.72	5.63	5.27	4.80	3.78	2.62	1.29	5.72	0.00
D1S3783	5.42	5.34	5.00	4.55	3.58	2.48	1.21	5.42	0.00
D1S1167	3.31	3.26	3.04	2.76	2.16	1.49	0.76	3.31	0.00
D1S3784	6.02	5.93	5.55	5.06	3.99	2.77	1.37	6.02	0.00
D1S2707	5.42	5.34	5.00	4.55	3.58	2.48	1.21	5.42	0.00
D1S3785	6.02	5.93	5.55	5.06	3.99	2.77	1.37	6.02	0.00
D1S3786	−∞	3.93	4.27	4.11	3.38	2.40	1.21	4.27	0.05
D1S484	−∞	3.34	3.72	3.59	2.98	2.11	1.05	3.72	0.05

and maintenance of the fluid and electrolyte balance. *CASQ1* has 11 exons¹⁵ and codes for calsequestrin, a calcium binding protein found in the cytoplasm of outer hair cells where it is thought to store and release calcium from membrane bound intracellular storage sites.¹⁶ Calcium is believed to play a major signalling role in outer hair cells by controlling metabolism, cytoskeletal integrity, cell shape, and cell excitability. *KCNJ10* consists of two exons¹⁷ and encodes an inwardly rectifying K⁺ channel subunit that is strongly expressed in the cochlear stria vascularis, where it is crucially involved in the generation of the endocochlear potential,^{18, 19} and in the satellite cells that surround the neurones and axons of the cochlear and vestibular ganglia.²⁰ Knockout mice for *KCNJ10* present a profound deafness and severe structural degeneration of the cochlea.^{21, 22} *KCNJ9* has three exons²³ and codes for a K⁺ channel with structural and functional similarity to *KCNJ10*,²⁴ although its expression has not been reported in the inner ear so far.

The exons and flanking regions of the four genes were investigated by heteroduplex and DNA sequencing in two affected subjects (I:1 and II:2). These analysis only revealed the non-pathogenic polymorphisms, 1097T→C in exon 3 of *KCNJ9* (NCBI SNP cluster id: rs3001040) and the IVS1-84T→C intronic change in *KCNJ10*, which was present in several control individuals with normal hearing. This last polymorphism allowed us to verify the presence in affected subjects of the two intact copies of *KCNJ10* exon 2, which include the entire coding region, so excluding the deletion of, or insertions in, this exon as the cause of deafness (data not shown).

Reports of more families with hearing deficit linked to DFNA49 may enable a further refinement of the critical interval facilitating the identification of the responsible gene. We are now searching for novel candidate genes at the DFNA49 interval.

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Authors' affiliations

M Angel Moreno-Pelayo, S Modamio-Høybjør, A Mencía, I del Castillo, F Moreno, Unidad de Genética Molecular, Hospital Ramón y Cajal, Madrid, Spain

S Chardenoux, C Petit, Unité de Génétique des Déficiences Sensoriels, CNRS URA 1968, Institut Pasteur, Paris, France

M Fernández-Burriel, Laboratorio de Genética Molecular, Hospital Universitario Materno Infantil, Las Palmas de Gran Canaria, Spain
M Lathrop, Centre National de Génotypage, Evry, France

*The first two authors contributed equally to this work and the order of authorship is arbitrary.

Correspondence to: Dr F Moreno, Unidad de Genética Molecular, Hospital Ramón y Cajal, Carretera de Colmenar Km 9, 28034, Madrid, Spain; fmoreno.hrc@salud.madrid.org

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ECHO.....

Susceptibility to Crohn's disease may lurk in enzyme mutation



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A preliminary study of genetic polymorphisms affecting transforming enzymes in the gut has singled out for the first time microsomal epoxide hydrolase as a likely candidate for susceptibility to Crohn's disease (CD).

Homozygous Tyr 113 substitution for His 113 in exon 3 of the microsomal epoxide hydrolase (*EPXH*) gene was the only one of seven variants in a series of genes coding for detoxifying enzymes with a significantly higher frequency in patients with CD than controls (47% v 21%, respectively). Tyr/Tyr genotype was also more common within the patient group than in the control group (allele frequency 0.67 v 0.61, respectively), and the odds of having the Tyr 113 allele were almost three times higher. This variant and another, in exon 4 of the same gene, were not associated with disease site, disease onset, fistulas, or history of bowel resection.

The variants were identified by PCR-RFLP (restriction fragment length polymorphism). They included those in cytochrome *P-450* 1A1C (*CYP1A1* 3' flanking region, *CYP1A1* exon 7); glutathione S-transferases mu-1, pi-1, and theta-1 (*GSTM1*, *GSTP1*, *GSTT1*); and epoxide hydrolases (*EPXH*) in exons 3 and 4. Screening was performed on 151 consecutive outpatients at a hospital clinic in the Netherlands and age and sex matched healthy controls; all were Caucasian.

Reactive oxygen species and their toxic metabolites have been implicated in inflammation of the gut in CD. Detoxifying enzymes, such as glutathione S-transferases and epoxide hydrolases, may also have a role, so polymorphisms affecting their activity may affect risk of developing CD.

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