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ORIGINAL INVESTIGATION

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A novel locus for autosomal dominant nonsyndromic hearing loss (DFNA44) maps to chromosome 3q28–29

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Abstract Hereditary non-syndromic sensorineural hearing loss (NSSHL) is a genetically highly heterogeneous group of disorders. Autosomal dominant forms account for up to 20% of cases. To date, 39 loci have been identified by linkage analysis of affected families that segregate NSSHL forms in the autosomal dominant mode (DFNA). Investigation of a large Spanish pedigree with autosomal dominant inheritance of bilateral and progressive NSSHL of postlingual onset excluded linkage to known DFNA loci and, in a subsequent genome-wide scan, the disorder locus was mapped to 3q28–29. A maximum two-point LOD score of 4.36 at θ=0 was obtained for marker D3S1601. Haplotype analysis placed the novel locus, DFNA44, within a 3-cM genetic interval defined by markers D3S1314 and D3S2418. Heteroduplex analysis and DNA sequencing of coding regions and exon/intron boundaries of two genes (CLDN16 and FGF12) in this interval did not reveal disease-causing mutations.

Introduction

Hearing loss is the most common sensory defect in humans. About 1/1000 children are affected by severe or profound sensorineural hearing impairment before speech acquisition (prelingual deafness) and genetic causes are estimated to be responsible for up to 60% of these cases (Morton 1991; Marazita et al. 1993). On the other hand, postlingual hearing loss is much more frequent, affecting 10% of the population by age 60 and 50% by age 80, resulting in the progressive social isolation of the affected individual (Petit 1996; Davis 1989). Most cases of postlingual deafness have a multifactorial etiology that results from a combination of genetic and environmental factors but monogenic forms exist that follow a mainly autosomal dominant mode of inheritance, representing about 10–20% of all cases of hereditary non-syndromic sensorineural hearing loss (NSSHL). Hereditary postlingual hearing loss is usually moderate to severe and progressive and often affects a particular range of frequencies (Bom et al. 1999). So far, more than 70 loci for non syndromic hereditary deafness have been described. Among them, 39 loci (DFNA1-DFNA30, DFNA32-DFNA38, DFNA40 and DFNA41) have been identified in familial cases with autosomal dominant sensorineural hearing loss (ADNSSHL) and 17 genes have been cloned (Van Camp and Smith 2002). In this study, we have identified a novel DFNA locus on chromosome 3q28–29 in a Spanish family with postlingual and progressive hearing loss.

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Materials and methods

Nomenclature

Gene symbols used in this article follow the recommendations of the HUGO Gene Nomenclature Committee (Povey et al. 2001).

Family data

A five-generation family (S281) with a history of ADNSSHL was ascertained through the Hospital Universitario "Puerta del Mar", Cádiz, in southern Spain. It consists of 40 members, 18 of whom are affected (Fig. 1). Appropriate informed consent was obtained from all study participants. Clinical evaluation was performed and blood samples were collected from 27 family members. DNA was extracted by standard techniques. Environmental factors were ex-

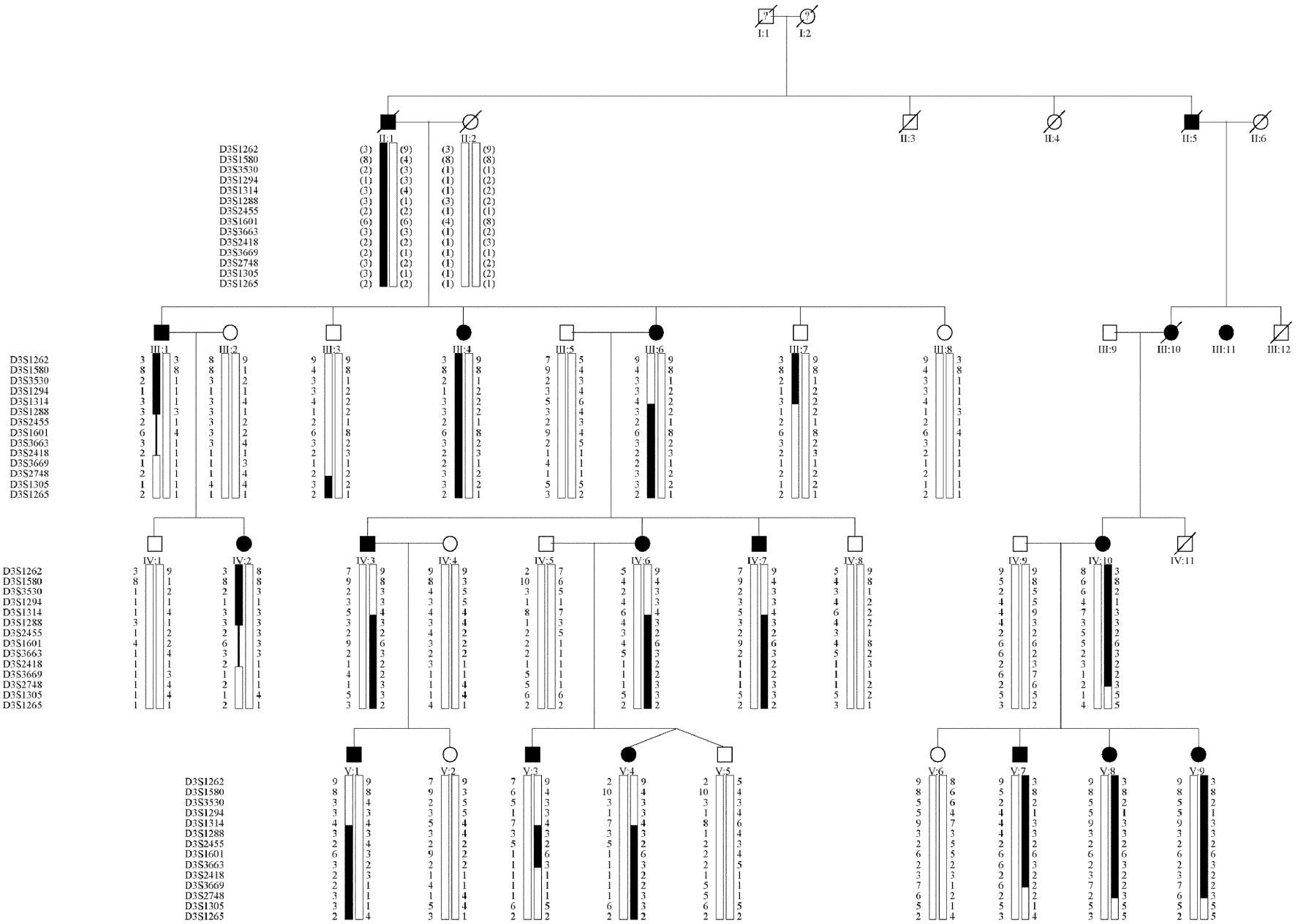

Fig. 1 Pedigree and haplotype analysis of the Spanish family S281. *Black symbols* represent affected subjects. Haplotypes are represented by *bars*, with the haplotype associated with hearing loss in *black*. A *thin line* between two segment bars indicates that the marker was not informative for mapping the recombination break point

cluded as the cause of hearing impairment. No syndromic features were present. Otoscopic examination and the use of tuning fork tests ruled out conductive hearing loss. Pure-tone audiometry was performed to test for air conduction (125–8000 Hz) and bone conduction (250–4000 Hz). Previous audiograms from patients were obtained when possible.

Genotyping and linkage analysis

A genome-wide screening was performed with 394 microsatellite markers distributed at intervals of, on average, 10 cM (ABI Prism Linkage Mapping Set 2, Applied Biosystems). Markers for the exclusion of all known DFNA loci and for the refinement of the critical interval were taken from the Généthon human linkage map (Dib et al. 1996) and from the Marshfield chromosome 3 map (http://research. marshfieldclinic.org /genetics). The order of the markers was established by integrating genetic and physical maps (NCBI, http://www.ncbi.nlm.nih.gov).

Fluorescently labelled alleles were analysed in an ABI PRISM 310 automated DNA sequencer (Applied Biosystems, Foster City, Calif.). Linkage analysis was performed by using the LINKAGE 5.1 software package (Lathrop et al. 1985). 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 as being equal to each other.

Candidate gene analysis

Primers were designed for the amplification of coding regions and exon-intron boundaries of CLDN16 (MIM 603959) and FGF12 (MIM 601513). Polymerase chain reaction (PCR) was performed by standard procedures as previously described (del Castillo et al. 2002). Heteroduplex analysis was carried out in MDE gels (BioWhittaker, Rockland, Me.) according to manufacturer's protocol. Sequences of PCR products were analysed in an automated DNA sequencer ABI PRISM 310 (Applied Biosystems).

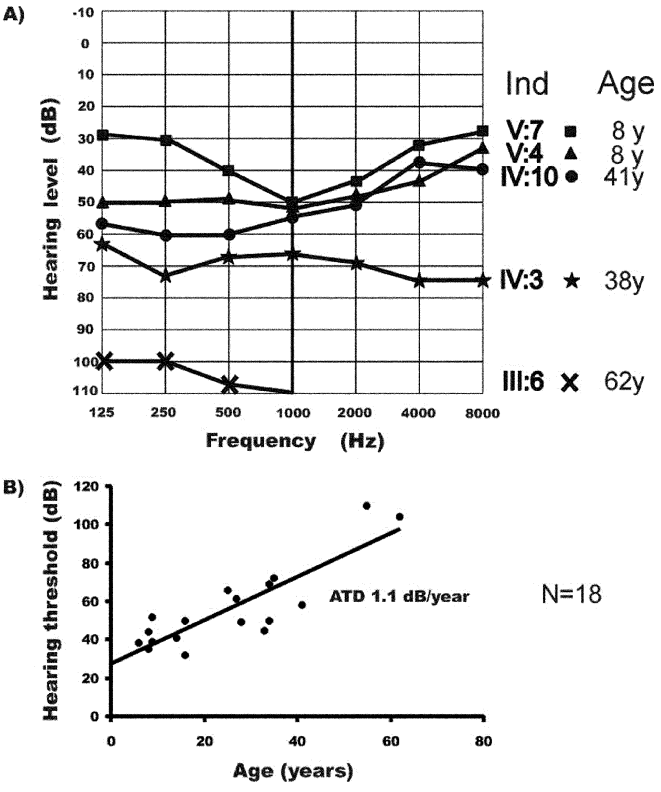

Fig. 2 **A** Audiograms showing the air conduction values obtained from five different affected subjects. Each graph point represents the average hearing loss for the right and left ears. Younger members show decreased hearing particularly at low-mid frequencies, whereas older subjects present a flat audiometric profile. **B** Plot depicting average hearing loss values (obtained from all available audiograms) versus age. Progression of the hearing loss was calculated by linear regression and expressed as annual threshold deterioration of all frequencies in dB per year (*ATD*)

Results and discussion

Clinical features

Affected subjects show bilateral, symmetrical and progressive sensorineural hearing impairment. Initially, the hearing loss in this family is moderate, mainly affecting low-mid frequencies (125–2000 Hz); it later progresses, involving all the frequencies (flat audiometric profile), to a profound hearing loss in the sixth decade (Fig. 2A). Linear regression analysis, based on all available audiograms from affected subjects, showed a 1.1 dB/year age-linked progression of the 125–8000 Hz average hearing loss (Fig. 2B). The onset of hearing loss was reported to occur in the first decade of life (6–10 years of age) in affected members of the family. No evidence of either vestibular dysfunction or occasional tinnitus was reported in affected subjects of the family. Computer tomography scan analysis of affected member III:1 ruled out inner ear malformations.

Linkage analysis

Twenty-six members of the family were considered informative for linkage. Subject V:2 is only 6 years old, below the age of onset, and therefore she was not included in the linkage analysis.

Initially, we tested the family for linkage to all described loci responsible for ADNSSHL. In all cases, negative results were obtained indicating the involvement of a novel locus responsible for the hearing loss in this family. Therefore, a genome-wide scan was performed with a set of 394 microsatellite markers, with an average spacing of 10 cM. Evidence of linkage was found to marker D3S1601 (maximum two-point LOD score of 4.36 at θ=0.000) in chromosomal region 3q28–29. The family was then genotyped for additional markers flanking D3S1601 in order to confirm linkage and for fine mapping of the genetic interval, resulting in significant LOD scores at θ=0 for markers D3S1288, D3S2455 and D3S3663 (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 position of the novel deafness locus, DFNA44, was delimited by haplotype analysis. This analysis revealed two key recombination events between D3S1314 and D3S1288 in individuals III:6 and III:7 on the centromeric side locating DFNA44 telomeric to marker D3S1314, and a crossover between D3S3663 and

Table 1 Two point Lod Scores between 3q28 microsatellite markers and DFNA44

Marker	Recombination fractions (θ):							Z_{max}	θ_{max}
	0.00	0.01	0.05	0.10	0.20	0.30	0.40		
D3S1262	–∞	1.49	1.94	1.94	1.60	1.11	0.55	1.97	0.07
D3S1580	–∞	–1.68	0.14	0.69	0.87	0.65	0.32	0.88	0.18
D3S3530	–∞	–0.18	1.55	1.98	1.88	1.34	0.61	2.03	0.13
D3S1294	–∞	0.81	1.88	2.06	1.77	1.18	0.52	2.06	0.10
D3S1314	–∞	1.96	2.98	3.08	2.61	1.81	0.85	3.09	0.09
D3S1288	3.82	3.75	3.46	3.09	2.30	1.45	0.56	3.82	0.00
D3S2455	3.94	3.87	3.59	3.22	2.47	1.65	0.77	3.94	0.00
D3S1601	4.36	4.28	3.98	3.59	2.77	1.89	0.92	4.36	0.00
D3S3663	4.20	4.13	3.83	3.44	2.63	1.76	0.80	4.20	0.00
D3S2418	–∞	1.38	1.84	1.84	1.51	1.02	0.45	1.87	0.07
D3S3669	–∞	1.06	2.11	2.26	1.90	1.21	0.43	2.27	0.09
D3S2748	–∞	–0.31	1.42	1.85	1.75	1.21	0.51	1.90	0.13
D3S1305	–∞	–5.06	–1.85	–0.70	0.08	0.23	0.14	0.23	0.30
D3S1265	–∞	–0.73	0.46	0.79	0.84	0.60	0.25	0.87	0.16

D3S2418 in individual V:3, on the telomeric side, mapping DFNA44 centromeric to D3S2418. Thus, DFNA44 lies within a critical interval of about 3 cM between marker D3S1314 on the proximal side and D3S2418 on the distal side (Fig. 1).

Candidate gene analysis

The DFNA44 critical interval established above spans a physical distance of about 2 Mb. Few known genes (CLDN16, IL1RAP and FGF12) have so far been identified in this interval (Fig. 3). No genes for syndromes involving hearing impairment have been mapped to this chromosome 3 region. The human chromosome region containing DFNA44 shares conserved synteny with a fragment of mouse chromosome 16 but no mutation causing hearing impairment in mouse mutants has been mapped to this region. Based on a candidate gene approach, CLDN16 (MIM603959) and FGF12 (MIM601513) were selected to be studied. The first gene, CLDN16, that codes for the tight junction protein claudin-16 (Simon et al. 1999) was considered a putative candidate since: (1) another claudin family member, claudin-14, is responsible for a recessive form of deafness, DFNB29 (Wilcox et al. 2001) and (2) tight junctions play a major role in the cochlea, compartmentalising endolymph and providing structural support for the auditory neuroepithelium (Furuse et al. 1998). Heteroduplex and sequence analysis of the five exons from CLDN16 only revealed an insertion/deletion mutation (165_166delGGinsC). This frameshift mutation would generate a premature TGA stop codon in exon 1. This change was detected in heterozygosity only in one affected subject, III:1, and in two normally hearing members, III:8 and IV:1, and so it was not considered to be responsible for the hearing loss, as it did not segregate with the disorder in this family. This complex mutation had been first reported in homozygosity in recessive forms of familial hypomagnesaemia with hypercalciuria and nephrocalcinosis resulting in renal failure (Weber et al. 2000). In the present family

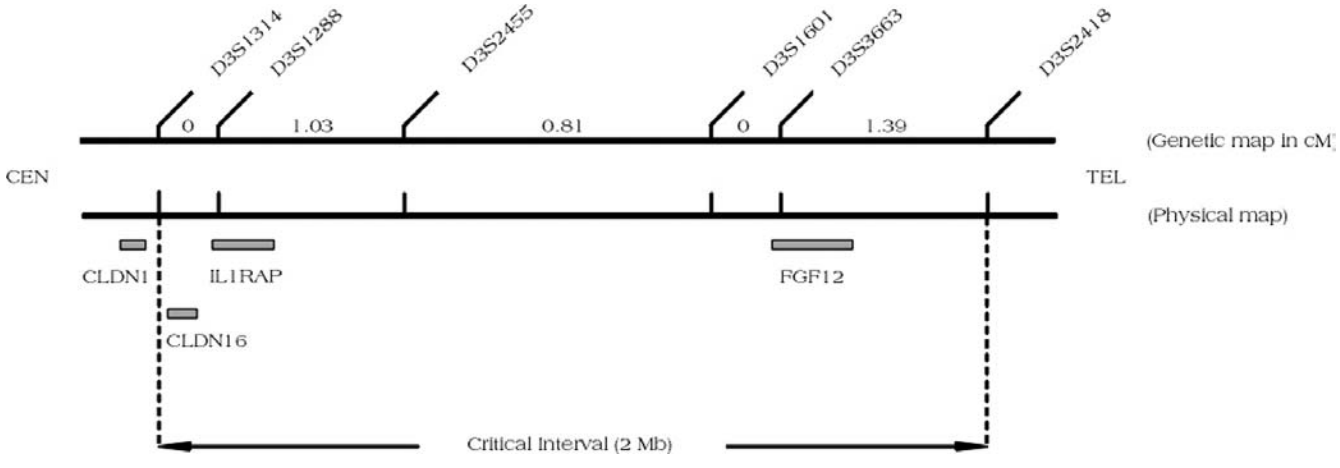

Fig. 3 Physical and genetic maps of the DFNA44 critical interval. *CEN* centromere, *TEL* telomere, *CLDN1* claudin 1 (MIM 603718), *CLDN16* claudin 16 (MIM 603959), *IL1RAP* interleukin 1 receptor associated protein (MIM 602626), *FGF12* fibroblast growth factor 12 (MIM 601513)

no signs of kidney disease have been reported, so the subjects III:1, III:8 and IV:1 seem to be just coincidental carriers of this mutation.

FGF12 (also known as FHF-1) is a fibroblast growth factor (FGF) homologue involved in nervous system development (Smallwood et al. 1996). Although its expression in the inner ear has not been reported so far, several FGF family members have major roles at different stages in inner ear development (Pickles et al. 2002), thus making FGF12 a candidate. FGF12 is encoded by five exons. Two FGF12 human isoforms have been described, which are generated through the use of two alternative transcription start sites (Muñoz-Sanjuan et al. 2000). Heteroduplex and sequence analysis of both isoforms did not reveal the disease-causing mutation.

Additionally, in order to find further putative candidates in the critical interval, inner ear expressed sequence tags (ESTs) were sought in the Morton Cochlear EST Database (http://hearing.bwh.harvard.edu/cochlearcdnalibrary.htm). Subsequent BLAST analysis rendered no matches within the DFNA44 interval. The critical interval also contains several poorly characterised genetic locations (LOC), with hypothetical homology to known genes, and several predicted genes of unknown function (NCBI resources), so progress in identifying the DFNA44 gene will depend on the isolation of candidate cDNAs from the critical interval.

The addition of this novel locus responsible for autosomal dominant hearing impairment to the broad catalogue of previously described loci supports the vast genetic heterogeneity underlying this disorder. The recombination events in this family have allowed us to narrow the critical interval to 3 cM, which should facilitate the identification of the responsible gene. It should also contribute further to our understanding of the inner ear.

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