Electronic letter

A cysteine substitution in the zona pellucida domain of α-tectorin results in autosomal dominant, postlingual, progressive, mid frequency hearing loss in a Spanish family

Miguel Angel Moreno-Pelayo, Ignacio del Castillo, Manuela Villamar, Lourdes Romero, Francisco Javier Hernández-Calvín, Carlos Herraiz, Rafael Barberá, Cristina Navas, Felipe Moreno

So far, 37 different loci for autosomal dominant, sensorineural, non-syndromic hearing loss (ADSNSHL), have been mapped and 11 genes have been cloned.[1] Among them, the *TECTA* gene, mapped to chromosome 11q22-24 (locus *DFNA8/A12*), encodes α-tectorin,[2] a non-collagenous component of the cochlear tectorial membrane. This membrane is an extracellular matrix that covers the apical surface of the sensory epithelium in the cochlea and plays an important role in transmitting the mechanical energy of sound to the mechanosensitive stereociliary bundles of the hair cells, where the sound is transduced into neural potentials. In previous studies, five different missense mutations resulting in ADSNSHL have been described in the *TECTA* gene in four *DFNA8/A12* families, affecting different domains of the protein and showing different phenotypes (table 1): a prelingual, non-progressive hearing loss affecting mid frequencies or a postlingual, progressive, high frequency hearing impairment.

In this study, we report a Spanish family with a novel phenotype of postlingual, progressive, mid frequency hearing loss resulting from a new mutation in the *TECTA* gene.

Unidad de Genética Molecular, Hospital Ramón y Cajal, Carretera de Colmenar Km 9, 28034 Madrid, Spain
M A Moreno-Pelayo
I del Castillo
M Villamar
L Romero
F Moreno

Servicio de ORL, Centro Nacional de Especialidades Quirúrgicas, Pabellón 8, Ciudad Universitaria, Madrid, Spain
F J Hernández-Calvín
C Herraiz

Servicio de ORL, Hospital Ramón y Cajal, Madrid, Spain
R Barberá
C Navas

Correspondence to: Dr Moreno, fmoreno@hrc.insalud.es

Materials and methods

The family consists of 47 members including nine affected subjects with ADSNSHL (fig 1A). Appropriate informed consent was obtained from all those studied. Clinical examination was performed and blood samples were obtained from 33 family members. Environmental factors were eliminated as the cause of deafness in all affected family members. Features suggestive of syndromic anomalies were not present. Otoscopic examination and use of the tuning fork test ruled out conductive hearing loss. Pure tone audiometry was performed to test for air conduction (frequencies of 125-8000 Hz) and bone conduction (frequencies of 250-8000 Hz). Affected subjects showed bilateral sensorineural hearing impairment. In the beginning, the hearing loss in this family is mild, mainly affecting mid frequencies (500, 1000, and 2000 Hz) (fig 1B) and later it progresses to moderate-severe hearing loss involving all the frequencies. Linear regression analysis, based on all available audiograms from the nine patients, showed a 0.4 dB/year age linked progression of the 0.5-2 KHz average hearing loss. The onset of the hearing loss ranged from childhood (<9 years) in affected members III.2, III.3, IV.1, IV.7, and V.2 to the second decade in the remaining affected subjects. No evidence of vestibular dysfunction was observed, except in patient IV.10 who reported occasional dizziness. In addition, there were symptoms of occasional tinnitus in four of nine affected members (III.5, IV.5, IV.7, and IV.10).

The subjects studied were genotyped for microsatellite markers close to all the described DFNA loci. Linkage analysis was performed using the LINKAGE 5.1 software package,[6] setting the frequency of the deafness gene to 0.0001 and considering marker allele frequencies to be equal to each other.

Results

In all cases negative results for linkage were obtained except for six microsatellite markers close to the *DFNA8/A12* locus. A maximum lod score of 3.67 was shown for marker D11S4089

Table 1 TECTA mutations described in the DFNA8/A12 families

Family	*Protein mutation*	*Exon*	*Domain*	*Onset*	*Progression*	*Frequencies*	*References*
Belgian	L1820F G1824D	17	ZP*	Prelingual	Stable	Mid	2
Spanish	C1837G	17	ZP	Postlingual	Progressive	Mid	Present report
Austrian	Y1870C	18	ZP	Prelingual	Stable	Mid	2
French	C1619S	14	ZA†	Pre- or postlingual	Progressive	High	3
Swedish	C1057S	10	ZA	Postlingual	Progressive	High	4

*Zona pellucida domain.
†Zonadhesin-like domain.

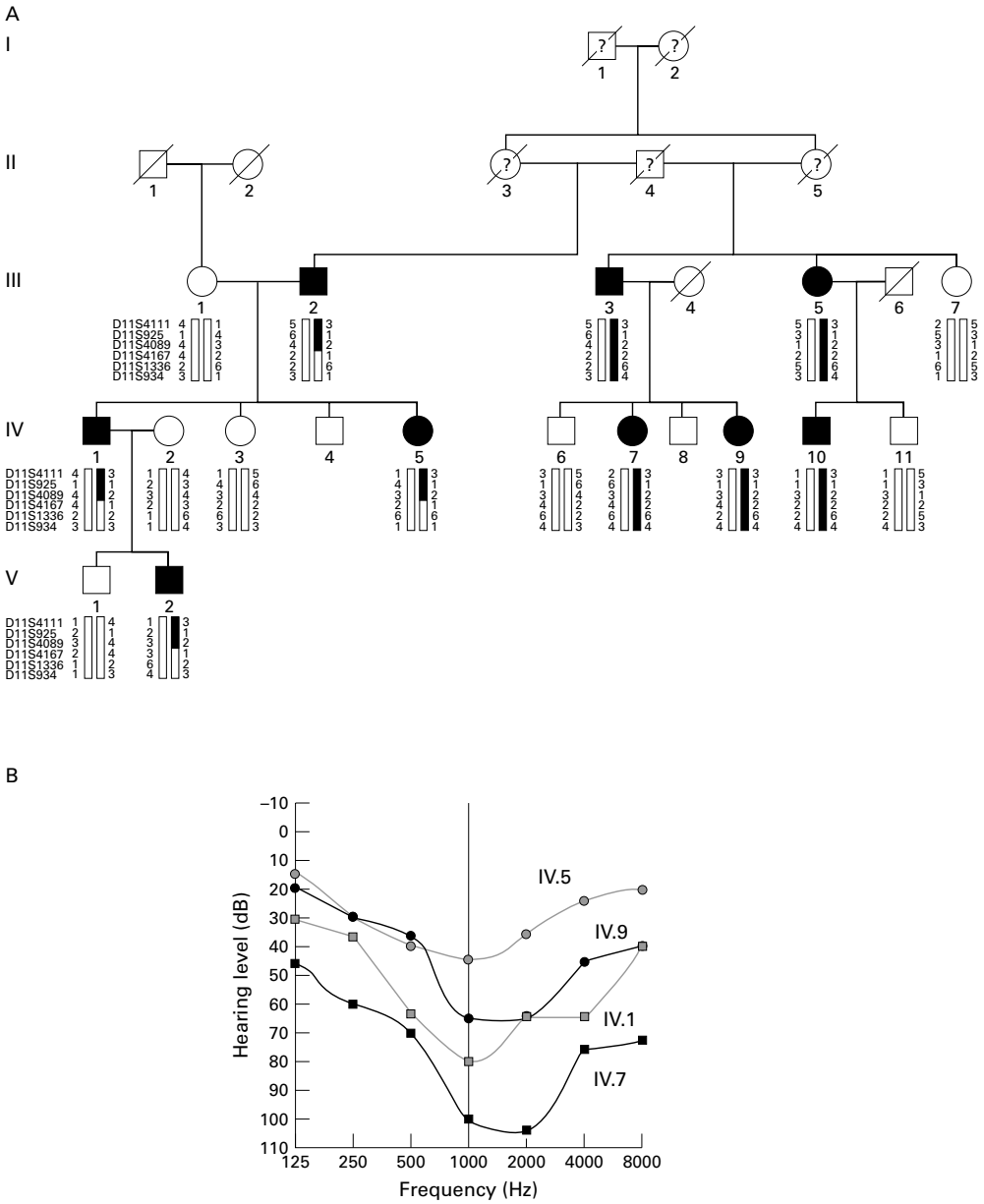

Figure 1 (A) Pedigree and haplotype analysis of the Spanish family S063. Only representative members have been included. The order of the markers was set integrating genetic and physical data from previous studies.[3 5] Black symbols represent affected subjects. Haplotypes are represented by bars, with the haplotype associated with hearing loss in black. (B) Audiograms from four different affected members of the family showing decreased hearing particularly at mid frequencies (500, 1000, and 2000 Hz). Only results for the right ear are presented.

at theta=0.0. Extensive alterations of the disease gene frequency, or of the allele frequencies of microsatellite markers, did not significantly change the lod scores. Detection of mutations in the *TECTA* gene was carried out by heteroduplex analysis followed by DNA sequencing of exons 10, 14, 17, 18, and the 5' end of intron 9 where all the previously reported mutations were located.[2–4 7] A novel mutation was detected in exon 17 of the *TECTA* gene. At nucleotide position 5509, a T to G transversion was found, which would produce a C1837G amino acid substitution (fig 2). This change results in the loss of the unique restriction site for enzyme *Alw*44I at exon 17. On this basis, we developed an easy screening test for the C1837G mutation. A 306 bp DNA fragment including exon 17 from the *TECTA* gene was amplified using specific primers designed at flanking intronic positions (forward primer: 5'-GAT TTG CCT TTC GTA ATA ACT GT-3', reverse primer: 5'-AGG ACA ATA AAT GTG CAA ACA CT-3'). After cleavage with the restriction enzyme *Alw*44I, two bands of 150 bp and 156 bp were seen in controls. In the affected members, an additional band corresponding to the 306 bp undigested product was also present. This missense mutation appeared in heterozygosity and was shown to segregate completely with the affected status in this family. In addition, it was not present in 100 unrelated Spanish controls.

Discussion

The α-tectorin precursor is proteolytically processed into three polypeptides: a module

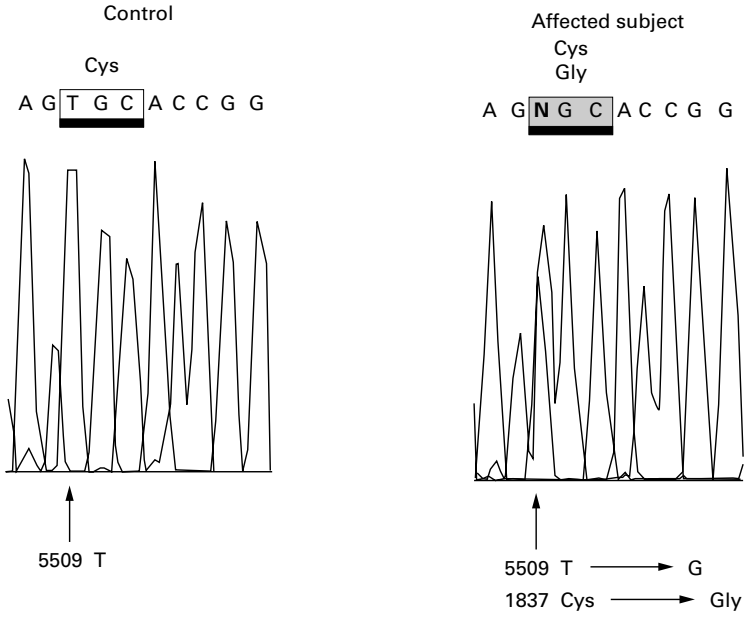

Figure 2 DNA sequences showing the TECTA missense mutation in the Spanish family S063. Electropherograms for the regions immediately surrounding the 5509T>G mutation at exon 17 are shown. An affected subject and a control are depicted. Codon 1837 is boxed.

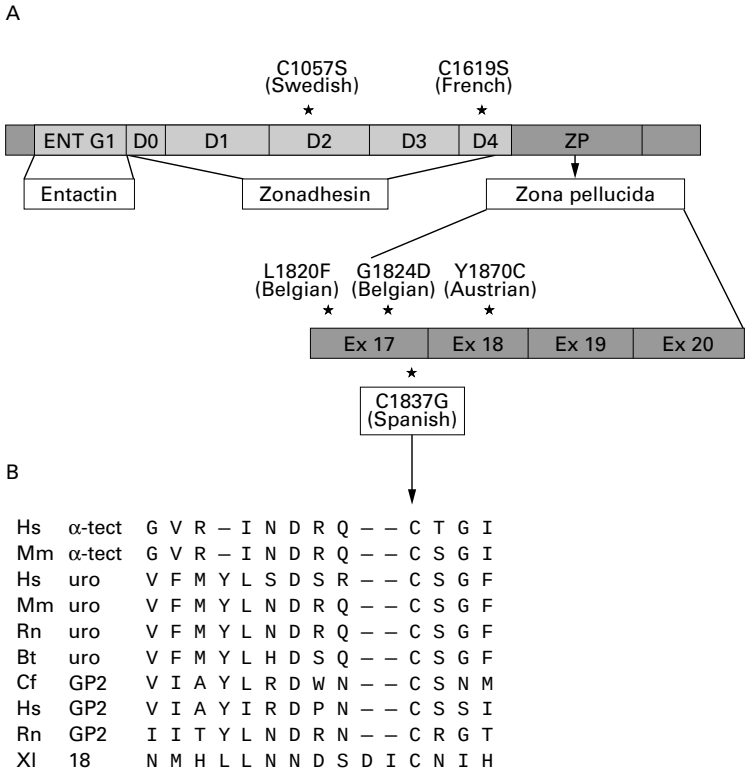

Figure 3 (A) Domain structure of the human α-tectorin protein. D0-D4 units represent the von Willebrand factor type D repeats (Zonadhesin-like). Asterisks indicate the position of the different missense mutations. The new C1837G mutation is boxed. (B) Multiple amino acid alignment of proteins homologous to the α-tectorin zona pellucida domain. Only a short region encoded by exon 17 and containing the C1837G mutation is shown. The alignment includes α-tectorin from human (Hs α-tect[2]) and mouse (Mm α-tect[13]), uromodulin from human (Hs uro[14]), mouse (Mm uro[15]), rat, and bovine (Rn uro and Bt uro[16]), glycoprotein 2 from dog (Cf GP2[17]), human (Hs GP2[18]), and rat (Rn GP2[19 20]), and frog thyroid regulated glycoprotein 18 (Xl 18[21]).

containing a region with similarity to the G1 domain of entactin,[8] a central module containing von Willebrand factor (vWf) type D repeats similar to zonadhesin (ZA),[9] and a module consisting of a zona pellucida domain (ZP).[10] It is assumed that these three polypeptides are cross linked to each other by disulphide bridges and interact with β-tectorin[11] to form the non-collagenous matrix of the tectorial membrane. The mutations in the Belgian and Austrian families change conserved amino acids from the ZP domain, whereas in the Swedish and French families cysteine residues from the ZA-like domain are substituted (table 1). The mutation in the French family changes one of the vicinal cysteine residues of the vWf-type D4 repeat that play an important role as a catalytic site for disulphide bonded multimer assembly of vWf.[12] In the Spanish family, the novel mutation is located in the ZP domain (fig 3A) and results in the replacement of a cysteine by a glycine. The cysteine residue at this position is fully conserved in all known ZP domains from other proteins (fig 3B), so the C1837G substitution might disrupt the proper interaction between the different tectorin polypeptides altering the mechanotransductional properties of the tectorial membrane leading to an inefficient transmission of sound. This hypothesis has also been proposed for the previously reported mutations in the *DFNA8/A12* families.[2–4]

It has been postulated that the phenotypic differences among the previously reported *DFNA8/A12* families (table 1) could be the result of the altered domain in α-tectorin in each family. Thus, mutations in the ZP domain appeared to lead to prelingual stable hearing loss mainly involving mid frequencies, while mutations in the ZA-like domain resulted in progressive hearing loss starting in the high frequencies.[2–4] The Spanish family reported here shows a novel phenotype not observed in the previously described *DFNA8/A12* families. The alteration of the ZP domain also affects mainly the mid frequencies in the beginning but in contrast it results in progressive hearing loss. This suggests that the alteration of one determined domain from α-tectorin determines the range of affected frequencies (ZA-like, high frequencies; ZP, mid frequencies) independently of the type of mutation. On the other hand, the progression or stability of the hearing loss seems to be related to the type of substituted residue in each domain. In particular, the substitution of the same type of residue (cysteine) in two different domains (ZA-like and ZP) appears to lead to a progressive and generally postlingual hearing loss that may be caused by a progressive deterioration of the tectorial membrane owing to the improper cross linking of the α-tectorin polypeptides. Identification of new mutations in additional families is needed to determine the validity of this putative genotype-phenotype correlation.

We are grateful to the Spanish family who made this research possible and Dr C Somoza-Castillo for patient referral. This work was supported by grants from the Fondo de Investigaciones Sanitarias FIS-96/1556, the Comisión de Investigación de Ciencia y Tecnología CICYT-SAF 99-0025, and the European Community QLRT 1999-00988.

1 Van Camp G, Smith RJH. Hereditary hearing loss homepage. World Wide Web URL: http: dnalab-www.uia.ac.be/dnalab/hhh
2 Verhoeven K, Van Laer L, Kirschhofer K, Legan PK, Hughes DC, Schatteman I, Verstreken M, Van Hauwe P,

Coucke P, Chen A, Smith RJ, Somers T, Offeciers FE, Van de Heyning P, Richardson GP, Wachtler F, Kimberling WJ, Willems PJ, Govaerts PJ, Van Camp G. Mutations in the human α-tectorin gene cause autosomal dominant non-syndromic hearing impairment. *Nat Genet* 1998;**19**:60-2.
3 Alloisio N, Morlé L, Bozon M, Godet J, Verhoeven K, Van Camp G, Plauchu H, Muller P, Collet L, Lina-Granade G. Mutation in the zonadhesin-like domain of α-tectorin associated with autosomal dominant non-syndromic hearing loss. *Eur J Hum Genet* 1999;**7**:255-8.
4 Balciuniene J, Dahl N, Jalonen P, Verhoeven K, Van Camp G, Borg E, Pettersson U, Jazin E. Alpha-tectorin involvement in hearing disabilities: one gene-two phenotypes. *Hum Genet* 1999;**105**:211-16.
5 Verhoeven K, Van Camp G, Govaerts PJ, Balemans W, Schatteman I, Verstreken M, Van Laer L, Smith RJ, Brown MR, Van de Heyning PH, Somers T, Offeciers FE, Willems PJ. A gene for autosomal dominant nonsyndromic hearing loss (DFNA12) maps to chromosome 11q22-24. *Am J Hum Genet* 1997;**60**:1168-74.
6 Lathrop GM, Lalouel JM, Julier C, Ott J. Multilocus linkage analysis in humans: detection of linkage and estimation of recombination. *Am J Hum Genet* 1985;**37**:482-98.
7 Mustapha M, Weil D, Chardenoux S, Elias S, El-Zir E, Beckmann JS, Loiselet J, Petit C. An alpha-tectorin gene defect causes a newly identified autosomal recessive form of sensorineural pre-lingual non-syndromic deafness, DFNB21. *Hum Mol Genet* 1999;**8**:409-12.
8 Durkin ME, Chakravarti S, Bartos BB, Liu SH, Friedman RL, Chung AE. Amino acid sequence and domain structure of entactin. Homology with epidermal growth factor precursor and low density lipoprotein receptor. *J Cell Biol* 1988;**107**:2749-56.
9 Hardy DM, Garbers DL. A sperm membrane protein that binds in a species-specific manner to the egg extracellular matrix is homologous to von Willebrand factor. *J Biol Chem* 1995;**270**:26025-8.
10 Bork P, Sander C. A large domain common to sperm receptors (Zp2 and Zp3) and TGF-beta type III receptor. *FEBS Lett* 1992;**300**:237-40.
11 Killick R, Legan PK, Malenczak C, Richardson GP. Molecular cloning of chick β-tectorin, an extracellular matrix molecule of the inner ear. *J Cell Biol* 1995;**129**:535-47.
12 Mayadas TN, Wagner DD. Vicinal cysteines in the prosequence play a role in the von Willebrand factor multimer assembly. *Proc Natl Acad Sci USA* 1992;**89**:3531-5.
13 Legan PK, Rau A, Keen JN, Richardson GP. The mouse tectorins. Modular matrix proteins of the inner ear homologous to components of the sperm-egg adhesion system. *J Biol Chem* 1997;**272**:8791-801.
14 Hession C, Decker JM, Sherblom AP, Kumar S, Yue CC, Mattaliano RJ, Tizard R, Kawashima E, Schmeissner U, Heletky S, Chow EP, Burne CA, Shaw A, Muchmore AV. Uromodulin (Tamm-Horsfall glycoprotein): a renal ligand for lymphokines. *Science* 1987;**237**:1479-84.
15 Prasadan K, Bates J, Badgett A, Dell M, Sukhatme V, Yu H, Kumar S. Nucleotide sequence and peptide motifs of mouse uromodulin (Tamm-Horsfall protein)-the most abundant protein in mammalian urine. *Biochim Biophys Acta*. 1995;**1260**:328-32.
16 Yu H, Papa F, Sukhatme VP. Bovine and rodent tamm-horsfall protein (THP) genes: cloning, structural analysis, and promoter identification. *Gene Expr* 1994;**4**:63-75.
17 Fukuoka S, Freedman SD, Scheele GA. A single gene encodes membrane-bound and free forms of GP-2, the major glycoprotein in pancreatic secretory (zymogen) granule membranes. *Proc Natl Acad Sci USA* 1991;**88**:2898-902.
18 Wong SM, Lowe AW. Sequence of the cDNA encoding human GP-2, the major membrane protein in the secretory granule of the exocrine pancreas. *Gene* 1996;**171**:311-12.
19 Hoops TC, Rindler MJ. Isolation of the cDNA encoding glycoprotein-2 (GP-2), the major zymogen granule membrane protein. Homology to uromodulin/Tamm-Horsfall protein. *J Biol Chem* 1991;**266**:4257-63.
20 Fukuoka S, Freedman SD, Yu H, Sukhatme VP, Scheele GA. GP-2/THP gene family encodes self-binding glycosylphosphatidylinositol-anchored proteins in apical secretory compartments of pancreas and kidney. *Proc Natl Acad Sci USA* 1992;**89**:1189-93.
21 Brown DD, Wang Z, Furlow JD, Kanamori A, Schwartzman RA, Remo BF, Pinder A. The thyroid hormone-induced tail resorption program during Xenopus laevis metamorphosis. *Proc Natl Acad Sci USA* 1996;**93**:1924-9.