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SHORT REPORT

Mutation in the zonadhesin-like domain of α-tectorin associated with autosomal dominant non-syndromic hearing loss

Nicole Alloisio[1], Laurette Morlé[1], Muriel Bozon[1], Jacqueline Godet[1], Kristien Verhoeven[2], Guy Van Camp[2], Henri Plauchu[3], Philippe Muller[4], Lionel Collet[5] and Geneviève Lina-Granade[5]

[1]*Centre de Génétique Moléculaire et Cellulaire, CNRS UMR 5534, Université Claude Bernard-Lyon I, Villeurbanne, France*
[2]*Department of Medical Genetics, University of Antwerp, Antwerp, Belgium*
[3]*Service de Génétique, Hospices Civils de Lyon, Hôtel-Dieu, Lyon*
[4]*Service d'ORL, Centre Hospitalier, Romans sur Isère*
[5]*Laboratoire Neurosciences et Systèmes Sensoriels, Département CNRS UPRESA 5020, d'Oto-Rhino-Laryngologie, Hôpital Edouard Herriot, Lyon, France*

A gene responsible for autosomal dominant non-syndromic hearing impairment in two families (DFNA8 and DFNA12) has recently been identified as *TECTA* encoding α-tectorin, a major component of the tectorial membrane. In these families, missense mutations within the zona pellucida domain of α-tectorin were associated with stable severe mid-frequency hearing loss. The present study reports linkage to DFNA12 in a new family with autosomal dominant high frequency hearing loss progressing from mild to moderate severity. The candidate region refined to 3.8 cM still contained the *TECTA* gene. A missense mutation (C1619S) was identified in the zonadhesin-like domain. This mutation abolishes the first of the vicinal cysteines (^{1619}Cys–Gly–Leu–^{1622}Cys) present in the D4 von Willebrand factor (vWf) type D repeat. These results further support the involvement of *TECTA* mutations in autosomal dominant hearing impairment, and suggest that vicinal cysteines are involved in tectorial membrane matrix assembly.

Keywords: hereditary deafness; hearing impairment; ear; cochlea; tectorial membrane; tectorin; vicinal cysteines; linkage analysis

Inherited non-syndromic hearing impairment constitutes about half the sensorineural hearing defect cases in children.[1,2] To date, 19 dominant (DFNA1 to 19), 20 recessive (DFNB1 to 20) and 8 X-linked (DFN1 to 8) loci have been reported.[3] Only eleven genes have so far been identified.[3] The DFNA12 locus was previously mapped to a 36 cM interval on chromosome 11q22–24 in a Belgian family.[4] The DFNA8 locus was assigned to chromosome 11q in an Austrian family.[3] Recently, the gene encoding α-tectorin (*TECTA*) was mapped within the above genetic interval.[5] Missense mutations within the zona pellucida domain of α-tectorin were identified

Correspondence: N Alloisio PhD, Génétique Humaine, CNRS UMR 5534, CGMC, Bâtiment 741, Université Lyon I, 43, boulevard du 11 Novembre 1918, 69622 Villeurbanne Cedex, France. Tel: 33 4 72 44 62 60; Fax: 33 4 72 44 05 55; E-mail: alloisio@univ-lyon1.fr
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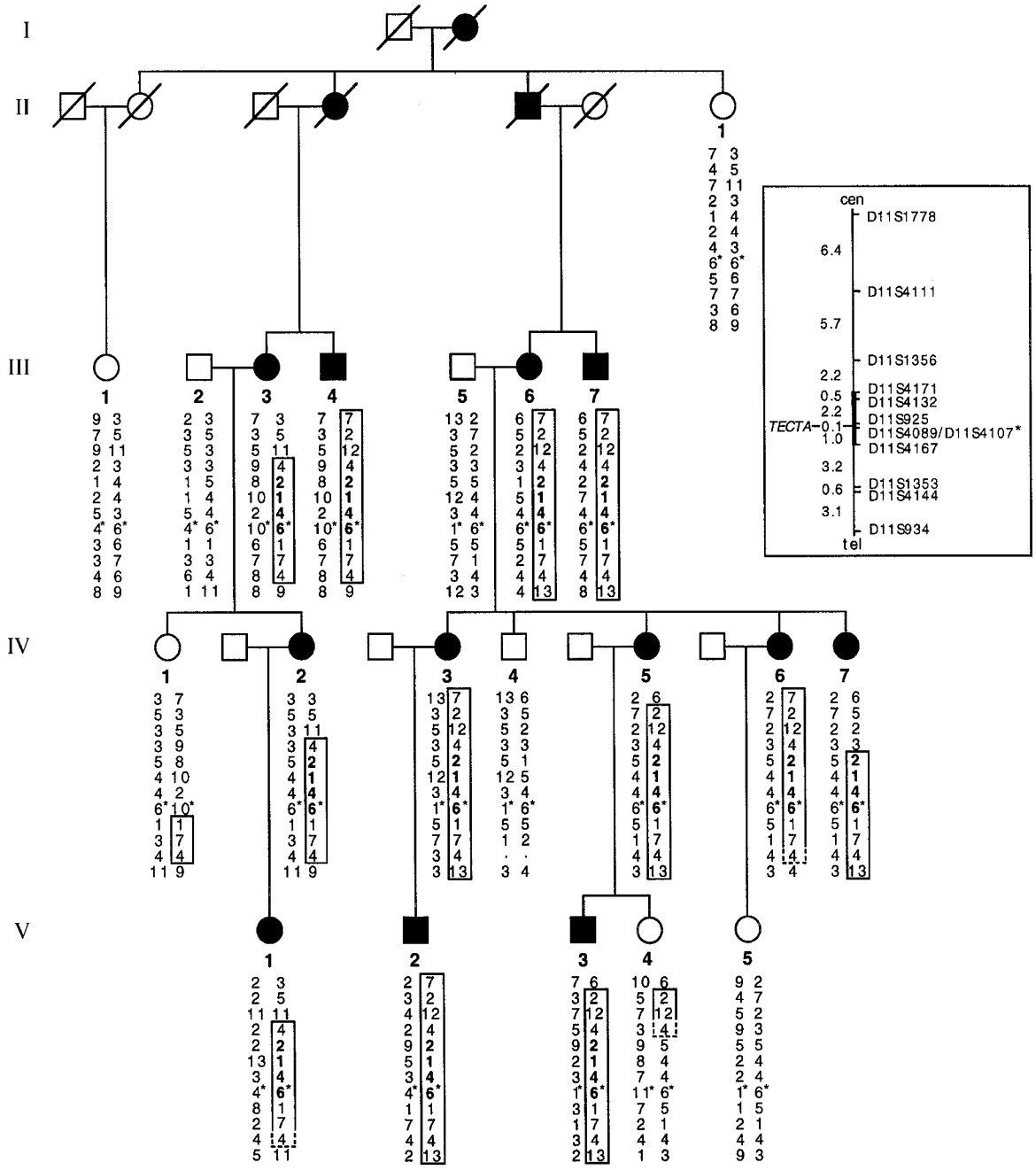

Figure 1 *Pedigree of the French family, showing haplotypes of chromosome 11 markers. Filled symbols denote affected individuals. Only family members included in the linkage study are numbered. The DFNA12-linked haplotype is boxed. Dashed lines indicate that the genotype is uninformative for those markers. Bold numbers indicate non-recombinant marker genotypes. Insert: Genetic distances (cM) between markers;*[13] *bold line: the 3.8 cM refined interval. Location of TECTA is indicated.*

in both these families.[6] The tectorial membrane, an extracellular matrix of the cochlea, plays a crucial role in the transmission of sound to the sensory hair cells. α- and β-tectorins interact with each other to form the filament-based non-collagenous matrix. An alteration of α-tectorin is likely to disrupt the structure of this matrix and, in consequence, to lead to inefficient transmission of sound.

The present French family (Figure 1) displayed autosomal dominant hearing impairment. In most of the 12

patients, hearing impairment was bilateral, symmetrical, cochlear, mild to moderate and predominating on high frequencies. It was detected before the age of 6 by subjective play-conditioned audiometry and transiently-evoked otoacoustic emission recording, performed as previously described.[7] Histories of delayed speech development suggested a prelingual onset in several patients. Linear regression analysis, based on all available audiograms from the 12 patients, showed a 0.7 dB/year age-linked progression of the 0.5–4 kHz average hearing loss. A bilateral vestibular dysfunction was suggested in patients IV.3, IV.5 and V2, who had started walking only at 24 months. Other patients' walking age was not known; none had any history of vertigo or dizziness.

Linkage analysis to known deafness loci[3] revealed a positive two-point lod score of 4.71 (at $\theta = 0$) for DFNA12 with marker D11S925, confirmed by additional markers (Figure 1). Combining information from nine recombinants, the candidate region was reduced to a 3.8 cM interval, still containing the *TECTA* gene. The presence of the known *TECTA* mutations was ruled out using specific restriction enzyme digestions.[6] A new mutation within the zona pellucida domain was also excluded by direct sequencing of exons 17 to 20. Single-strand conformation polymorphism screening of the 19 remaining exons was then conducted, as previously described.[3,6] Exon 14 analysis revealed additional bands in patients. Its direct sequencing identified a heterozygous G -> C missense mutation at nucleotide 4857 (Figure 2), which replaces the cysteine at residue 1619 with a serine (C1619S) and creates a new site for MspI. The C1619S mutation segregated in the 12 affected members and was not in any of the 8 unaffected members or in 100 French controls. No other variation was found in patients except for a silent mutation (4099G -> A; T1366T) in exon 11 which turned out to be a frequent polymorphism (9 out of 25 French controls were found to be heterozygotes).

The C1619S amino acid change abolishes the first of the vicinal cysteines (^{1619}CGL^{1622}C) present in the D4 vWf type D repeat of the zonadhesin-like domain.[6,8] Cysteine residues separated by two amino acids correspond to the catalytic site of protein disulphide isomerases.[9] The CGLC motif present in the D1 and D2 repeats of vWf has been shown to catalyse disulphide-bonded oligomerisation of vWf.[10] This motif is also found in D repeats of zonadhesin and intestinal mucin MUC2, which both exist as covalent oligomers.[11,12] Therefore the CGLC motif present in the D1 and D4

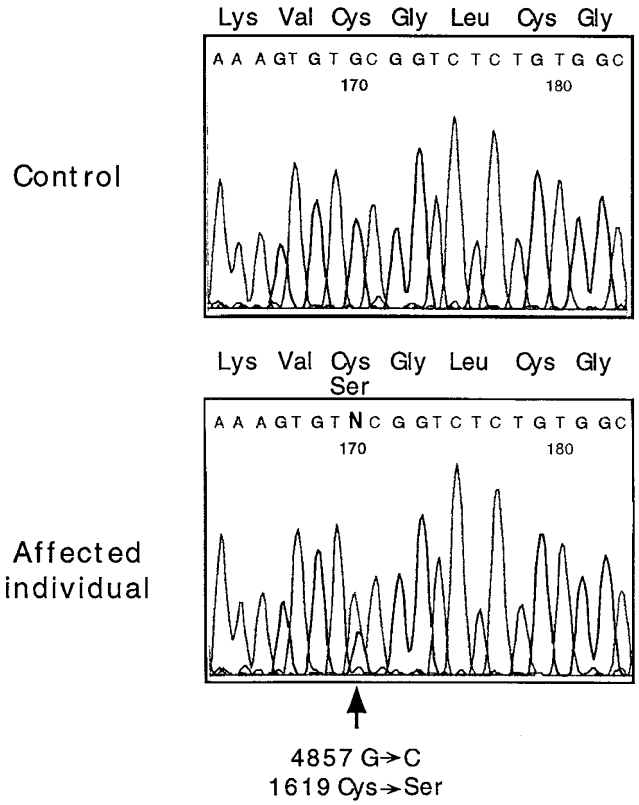

Figure 2 *DNA sequences showing the* TECTA *missense mutation in the French family. Exon 14 PCR-products were sequenced on an Applied Biosystems (ABI373) DNA sequencer.*

repeats of α-tectorin may be involved in some aspect of tectorial membrane matrix assembly.[6,8] However, at present, the respective roles which the three distinct domains composing α-tectorin may play in organising this filament-based matrix in conjunction with β-tectorin remain to be elucidated, and the effect of the C1619S substitution needs to be investigated.

This French family presented a progressive mild to moderate high-frequency hearing loss, whereas the Belgian and Austrian families[6] had stable, more severe, mid-frequency hearing loss. These phenotypic differences correspond to mutations located in two distinct domains which probably play different roles. The finding of additional mutations should allow such a genotype–phenotype relationship to be confirmed.

An early bilateral vestibular dysfunction suggested in some patients of the French family might be explained by α-tectorin role in the vestibular organ: α-tectorin is expressed in the 2-day mouse utricular and saccular maculae and thus might be a component of the otolithic membrane or of its accessory membrane.[8]

This study provides further evidence that mutations in *TECTA* cause autosomal dominant hearing impairment and suggests that vicinal cysteines play an important role in tectorial membrane structure.

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