 Human Molecular Genetics, 1999, Vol. 8, No. 3 **409–412**

ARTICLE

An α-tectorin gene defect causes a newly identified autosomal recessive form of sensorineural pre-lingual non-syndromic deafness, DFNB21

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Received October 13, 1998; Revised and Accepted December 1, 1998

In our efforts to identify new loci responsible for non-syndromic autosomal recessive forms of deafness, DFNB loci, we have pursued the analysis of large consanguineous affected families living in geographically isolated areas. Here, we report on the study of a Lebanese family comprising nine members presenting with a pre-lingual severe to profound sensorineural isolated form of deafness. Linkage analysis led to the characterization of a new locus, DFNB21, which was assigned to chromosome 11q23–25. Already mapped to this chromosomal region was *TECTA*. This gene encodes α-tectorin, a 2155 amino acid protein which is a component of the tectorial membrane. This gene recently has been shown to be responsible for a dominant form of deafness, DFNA8/12. Sequence analysis of the *TECTA* gene in the DFNB21-affected family revealed a G to A transition in the donor splice site (GT) of intron 9, predicted to lead to a truncated protein of 971 amino acids. This establishes that α-tectorin mutations can be responsible for both dominant and recessive forms of deafness. Comparison of the phenotype of the DFNB21 heterozygous carriers with that of DFNA8/12-affected individuals supports the hypothesis that the *TECTA* mutations which cause the dominant form of deafness have a dominant-negative effect. The present results provide genetic evidence for α-tectorin forming homo- or heteromeric structures.

INTRODUCTION

Approximately 1 in 1000 children is affected by deafness at birth or before 2 years of age, i.e. in the pre-lingual period. In the majority of cases, deafness is the sole symptom (non-syndromic deafness). In developed countries, two-thirds of pre-lingual non-syndromic deafness cases are estimated to be of a genetic origin. Among these forms, the autosomal recessive forms (DFNB) are the most frequent (80%) and the most severe. They are almost exclusively sensorineural due to a cochlear defect (1). To date, 20 DFNB loci have been reported (website http://hgins.uia.ac.be/dnalab/hhh/recessive.html); for four of them, the corresponding genes have been identified, namely the connexin 26 gene (*GJB2*) for DFNB1 (2), the myosin VIIA gene (*MYO7A*) for DFNB2 (3,4), the myosin XV gene (*MYO15*) for DFNB3 (5) and the pendrin gene (*PDS*) for DFNB4 (6) (for a review, see ref. 7). In order to identify novel DFNB loci, we pursued a systematic analysis of affected consanguineous families living in isolated regions around the Mediterranean sea. We report here on a new DFNB locus (DFNB21) and the identification of the causative gene.

RESULTS AND DISCUSSION

In family Z, living in the centre of Lebanon and belonging to the Chiite community, nine individuals were recognized as deaf in the pre-lingual period (Fig. 1). Pure-tone audiometry with a recording of pure-tone air and bone conduction thresholds and auditory brainstem response (ABR) analyses established that the affected individuals suffered from a severe or profound (70–110 dB of hearing loss on all frequencies) sensorineural form of deafness. The audiometric tests were normal for their parents. Genetic linkage analysis performed in the generation V children and their parents excluded the possible involvement of one of the 20 already known DFNB loci. A systematic genome screen was then undertaken. Linkage to the microsatellite polymorphic marker

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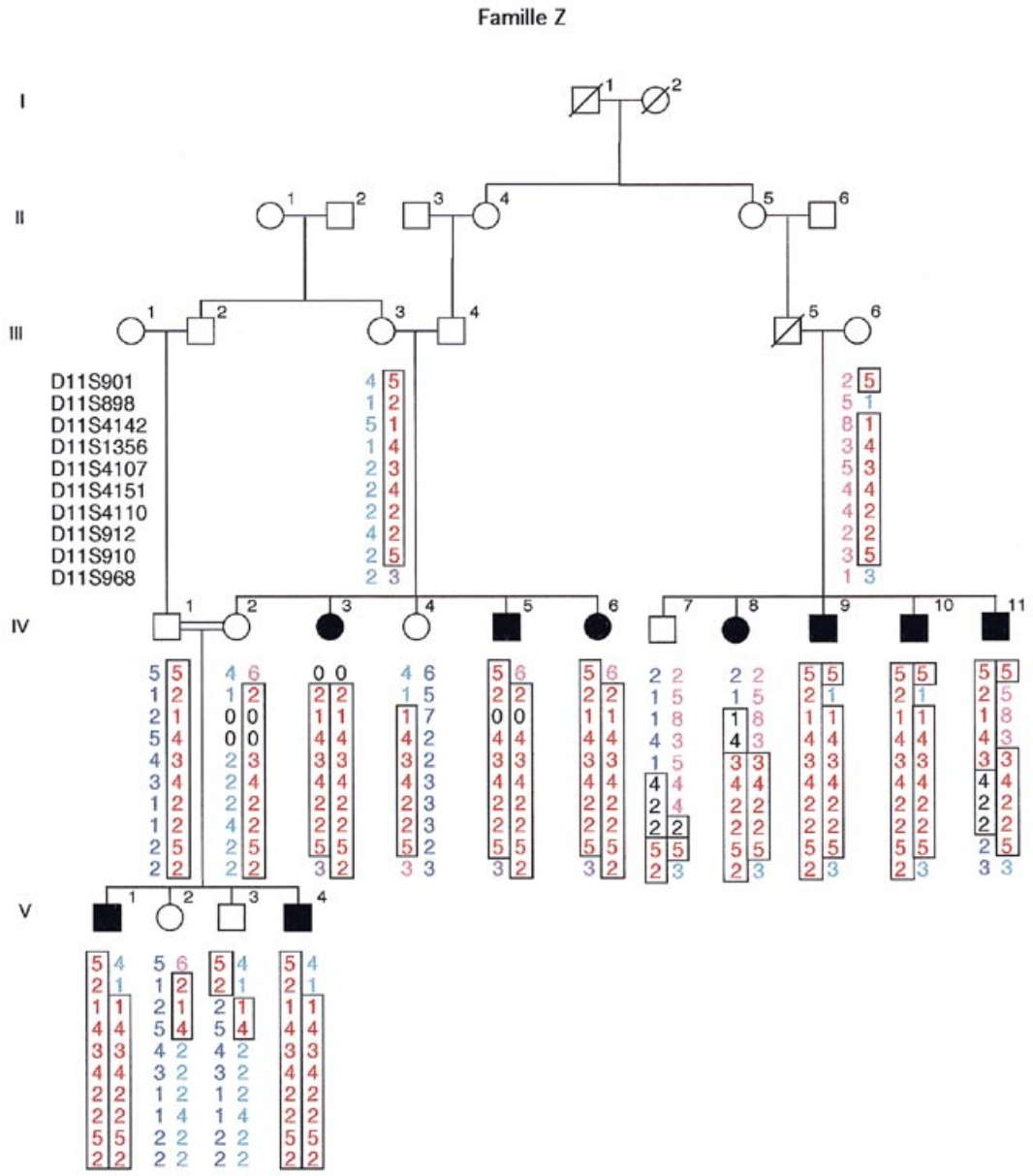

Figure 1. Segregation analysis of family Z affected by a non-syndromic sensorineural, autosomal recessive form of deafness, using AFM polymorphic markers in the 11q region (25). '0' indicates ambiguous positioning of the allele on the gel. Affected individuals are represented by solid symbols. The core haplotype associated with DFNB21 is boxed. *TECTA* is located between *D11S925* and *D11S4107* (12).

D11S4151 was detected. Segregation analysis with polymorphic markers of the corresponding chromosomal region resulted in a significant lod score of 5.92 (θ = 0.0) with *D11S4107* (see Materials and Methods). This identified a novel locus for recessive deafness, DFNB21. By the analysis of other members of family Z, DFNB21 could thus be assigned to a 28 cM region of homozygosity at 11q23–25, delineated by the markers *D11S1356* and *D11S910*, which was shared by the nine deaf individuals (Fig. 1).

Two dominant forms of deafness (DFNA), which are now recognized as the same form, DFNA8/12, had been assigned to this chromosomal region (8,9), and the *TECTA* gene, encoding α-tectorin, a protein specifically expressed in the inner ear (10), recently has been shown to be the causative gene (11). This gene has been mapped between *D11S925* and *D11S4107* (12), i.e. within the candidate interval for DFNB21. Thus *TECTA* appeared an attractive candidate gene for DFNB21. The 23 coding exons of *TECTA* were PCR amplified in patient V-1 using the previously described primers (11), and sequenced. This led to the identification of a G→A transition in the donor splice site (GT) of intron 9 (Fig. 2). The mutation was homozygous in the nine affected individuals and heterozygous in their parents (four parents available for analysis). It was not detected in 101 normal hearing individuals living in Lebanon who were unrelated to this family.

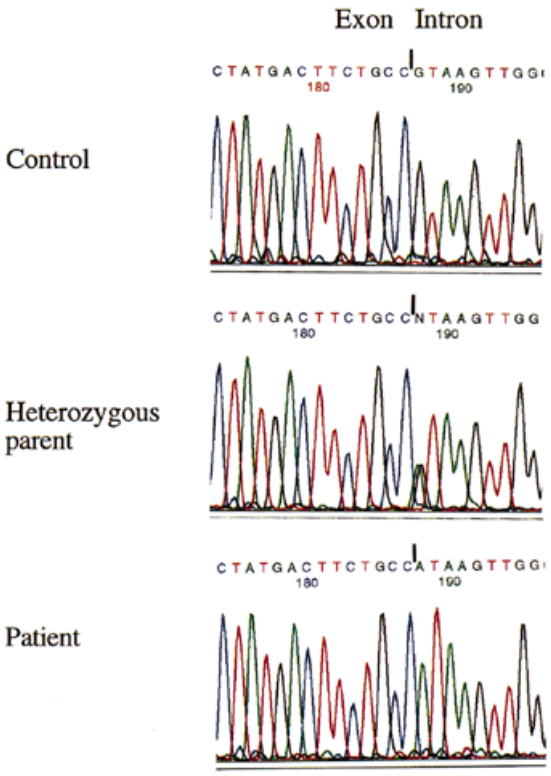

Figure 2. DNA sequencing of the 3′ end of the α-tectorin exon 9 and the adjacent intron of a control individual, a heterozygous parent and an affected individual showing the G→A transition in the donor splice site.

The effect of such mutations has been documented extensively in situations in which the transcripts of the mutated gene could be analysed directly, and was found to lead to the skipping of the preceding exon (13–16). Accordingly, in DFNB21 patients, the skipping of exon 9 would result in a premature stop codon at amino acid position 972. These results thus identify *TECTA* as underlying DFNB21.

The *TECTA*-encoded protein, α-tectorin, is one of the non-collagenous components of the tectorial membrane (10,17,18), the acellular matrix which covers the neuroepithelium of the cochlea. Upon sound stimulation, the relative displacement of the tectorial membrane with regards to the hair cells provokes a deflection of their stereociliary bundles, thereby leading to the opening of their mechanotransduction channel. α-Tectorin is a large modular protein of 2155 amino acids in humans. Its sequence analysis predicts a secreted molecule, synthesized as a lipid-linked membrane-bound precursor with a glycosylphosphatidylinositol anchorage, which subsequently is released from the membrane by a proteolytic cleavage. Several domains have been recognized in this protein. An N-terminal region homologous to the first globular domain (G1) of entactin is followed by three full repeats and two partial repeats homologous to the D domains of pre-pro-von Willebrand factor (vWF) and a C-terminal region containing a zona pellucida (ZP) domain. To date, mutations have been described in three DFNA8/12-affected families (Table 1). In these families, the degree of hearing loss severity varied from mild to moderately severe. In two of them, the reported mutations were missense mutations located in the ZP domain (11). In the third family, a missense mutation was observed in the fourth vWF type D domain (12); it substitutes a serine for the first cysteine of a CGLC motif which has been demonstrated to be involved in the disulfide-bonded oligomerization of the vWF.

Based on the normal auditory function of the heterozygous carriers of family Z, we propose that half of the normal amount of α-tectorin is sufficient to preserve the mechanical and electrical properties of the tectorial membrane (19). As a corollary, this leads us to conclude that the mutations in DFNA8/12 should have a dominant-negative effect. This, in turn, indicates that the mutated α-tectorin in DFNA8/12-affected individuals interacts with other molecules, i.e. normal α-tectorin, β-tectorin (10,17) or other components of the tectorial membrane (18). Along this line, the ZP domain which is known to be an interacting domain, is also present in β-tectorin and in numerous proteins associated with filaments or gels (20). The present results provide genetic evidence supporting the idea that α-tectorin is involved in homo- or heteromeric structures.

Table 1. Phenotype–genotype correlations in patients with *TECTA* mutations

Family (reference)	Degree of severity	Age of onset	Mutation	Protein modification
DFNA8 (8)	moderate–moderately severe	pre-lingual	5876A→G/exon 18	Y1870C
(8 affected)	60–80 dBHL			ZP domain
	all frequencies			
DFNA12 (9)	mild–moderately severe	pre-lingual or	5725C→T/exon 17	L1820F
(14 affected)	20–80 dBHL	early childhood	5738G→A/exon 17	G1824D
	mid-frequencies			ZP domain
DFNA8/12 (12)	mild–moderately severe	pre-lingual or	4857G→C/exon 14	C1619S
(12 affected)	20–80 dBHL	early childhood		D4 vWF type D repeat (10)
	high frequencies			
DFNB21 (this study)	moderately severe–profound	pre-lingual	G→A/intron 9 donor site	truncated protein
(9 affected)	70–110 dBHL		skipping of exon 9	stop codon in:
	all frequencies			D2 vWF type D repeat (10)

ZP, zona pellucida; vWF, von Willebrand factor.

The data presented here bring the number of genes underlying both DFNA and DFNB forms to three; the two other genes being *Cx26* for DFNB1 (2) and DFNA3 (21), and *MYO7A* for DFNB2 (3,4) and DFNA11 (22). For connexin 26 (23) and myosin VIIA (4), their multimeric structures have been established.

MATERIALS AND METHODS

Auditory tests

Pure-tone audiometry with air and bone conduction at 250, 500, 1000, 2000, 4000 and 8000 Hz was performed systematically (with a Beltone 2000 clinical audiometer), as well as otoscopic examinations, for each adult individual. In the young affected children, the ABR was recorded.

Genotyping of microsatellite markers

DNA extraction, genotyping and fluorescent microsatellite marker amplification were as previously reported (24). The sequences of the primers *D11S901*, *D11S898*, *D11S4142*, *D11S1356*, *D11S4107*, *D11S4151*, *D11S4110*, *D11S912*, *D11S910* and *D11S968* have been reported (25).

Linkage analysis

Linkage analysis was done using MLINK (V 5.2) in its FASTLINK implementation (V 4.0). The disease was assumed to be inherited in a recessive mode and fully penetrant.

Screening for mutations in the α-tectorin gene

The 23 exons of *TECTA* were amplified from genomic DNA, with the primers described elsewhere (11), and sequenced on an ABI 377 Perkin Elmer sequencer. The sequencing primer for exon 9 was 5′-GGTGCGGCATCATCAACGACC-3′.

ACKNOWLEDGEMENTS

We are grateful to the members of family Z for their participation, to Jean-Pierre Hardelin, Vasiliki Kalatzis and Jacqueline Levilliers for critical reading of the manuscript, to Fabienne Levi-Acobas for expert sequencing, and to N. Surin for helpful advice. This work was supported by grants from AFM and Association Entendre (France), University Saint Joseph (Lebanon) and EEC (BMH4-CT96-1324).

REFERENCES

1. Petit, C. (1996) Genes responsible for human hereditary deafness: symphony of a thousand. *Nature Genet*., **14**, 385–391.
2. Kelsell, D.P., Dunlop, J., Stevens, H.P., Lench, N.J., Liang, J.N., Parry, G., Mueller, R.F. and Leigh, I.M. (1997) Connexin 26 mutations in hereditary non-syndromic sensorineural deafness. *Nature*, **387**, 80–83.
3. Liu, X.-Z., Walsh, J., Mburu, P., Kendrick-Jones, J., Cope, M.J.T.V., Steel, K.P. and Brown, S.D.M. (1997) Mutations in the myosin VIIA gene cause non-syndromic recessive deafness. *Nature Genet*., **16**, 188–190.
4. Weil, D., Küssel, P., Blanchard, S., Lévy, G., Levi-Acobas, F., Drira, M., Ayadi, H. and Petit, C. (1997) The autosomal recessive isolated deafness, DFNB2, and the Usher 1B syndrome are allelic defects of the myosin-VIIA gene. *Nature Genet*., **16**, 191–193.
5. Wang, A., Liang, Y., Fridell, R.A., Probst, F.J., Wilcox, E.R., Touchman, J.W., Morton, C.C., Morell, R.J., Noben-Trauth, K., Camper, S.A. and Friedman, T.B. (1998) Association of unconventional myosin *MYO15* mutations with human nonsyndromic deafness DFNB3. *Science*, **280**, 1447–1451.
6. Li, X.C., Everett, L.A., Lalwani, A.K., Desmukh, D., Friedman, T.B., Green, E.D. and Wilcox, E.R. (1998) A mutation in *PDS* causes non-syndromic recessive deafness. *Nature Genet*., **18**, 215–217.
7. Kalatzis, V. and Petit, C. (1998) The fundamental and medical impacts of recent progress in research on hereditary hearing loss. *Hum. Mol. Genet*., **7**, 1589–1597.
8. Kirschhofer, K., Kenyon, J.B., Hoover, D.M., Franz, P., Weipoltshammer, K., Wachtler, F. and Kimberling, W.J. (1996) Autosomal-dominant congenital severe sensorineural hearing loss. Localisation of a disease gene to chromosome 11q by linkage in an Austrian family. European Workgroup on Genetics of Hearing Impairment, Milan, Italy, October 11–13.
9. Verhoeven, K., Van Camp, G., Govaerts, P.J., Balemans, W., Schatteman, I., Verstreken, M., Van Laer, L., Smith, R.J.H., Brown, M.R., Van de Heyning, P.H., Somers, T., Offeciers, F.E. and Willems, P.J. (1997) A gene for autosomal dominant nonsyndromic hearing loss (DFNA12) maps to chromosome 11q22–24. *Am. J. Hum. Genet*., **60**, 1168–1174.
10. Legan, P.K., Rau, A., Keen, J.N. and Richardson, G.P. (1997) The mouse tectorins. Modular matrix proteins of the inner ear homologous to components of the sperm–egg adhesion system. *J. Biol. Chem*., **272**, 8791–8801.
11. Verhoeven, K., Van Laer, L., Kirschhofer, K., Legan, P.K., Hughes, D.C., Schatteman, I., Verstreken, M., Van Hauwe, P., Coucke, P., Chen, A., Smith, R.J.H., Somers, T., Offeciers, F.E., Van de Heyning, P., Richardson, G.P., Wachtler, F., Kimberling, W.J., Willems, P.J., Govaerts, P.J. and Van Camp, G. (1998) Mutations in the human α-tectorin gene cause autosomal dominant non-syndromic hearing impairment. *Nature Genet*., **19**, 60–62.
12. Alloisio, N., Morlé, L., Bozon, M., Godet, J., Verhoeven, K., Van Camp, G., Plauchu, H., Muller, P., Collet, L. and Lina-Granade, G. (1999) Mutation in the zonadhesin-like domain of α-tectorin associated with autosomal dominant non-syndromic hearing loss. *Eur. J. Hum. Genet*., in press.
13. Weil, D., Bernard, M., Combates, N., Wirtz, M.K., Hollister, D.W., Steinmann, B. and Ramirez, F. (1988) Identification of a mutation that causes exon skipping during collagen pre-mRNA splicing in an Ehlers–Danlos syndrome variant. *J. Biol. Chem*., **263**, 8561–8564.
14. Berg, L.P., Grundy, C.B., Thomas, F., Millar, D.S., Green, P.J., Slomski, R., Reiss, J., Kakkar, V.V. and Cooper, D.N. (1992) *De novo* splice site mutation in the antithrombin III (AT3) gene causing recurrent venous thrombosis: demonstration of exon skipping by ectopic transcript analysis. *Genomics*, **13**, 1359–1361.
15. Parkinson, D.B. and Thakker, R.V. (1992) A donor splice site mutation in the parathyroid hormone gene is associated with autosomal recessive hypoparathyroidism. *Nature Genet*., **1**, 149–152.
16. Vidal, F., Aberdam, D., Miquel, C., Christiano, A.M., Pulkkinen, L., Uitto, J., Ortonne, J.P. and Meneguzzi, G. (1995) Integrin beta 4 mutations associated with junctional epidermolysis bullosa with pyloric atresia. *Nature Genet*., **10**, 229–234.
17. Killick, R., Legan, P.K., Malenczak, C. and Richardson, G.P. (1995) Molecular cloning of chick β-tectorin, an extracellular matrix molecule of the inner ear. *J. Cell Biol*., **129**, 535–547.
18. Cohen-Salmon, M., El-Amraoui, A., Leibovici, M. and Petit, C. (1997) Otogelin: a glycoprotein specific to the acellular membranes of the inner ear. *Proc. Natl Acad. Sci. USA*, **94**, 14450–14455.
19. Steel, K.P. (1986) Tectorial membrane. In Altschuler, R.A., Hoffman, D.W. and Bobbin, R.P. (eds), *Neurobiology of Hearing: The Cochlea*. Raven Press, New York, pp. 139–147.
20. Greve, J.M. and Wassarman, P.M. (1985) Mouse egg extracellular coat is a matrix of interconnected filaments possessing a structural repeat. *J. Mol. Biol*., **181**, 253–264.
21. Denoyelle, F., Lina-Granade, G., Plauchu, H., Bruzzone, R., Chaïb, H., Levi-Acobas, F., Weil, D. and Petit, C. (1998) Connexin26 gene linked to a dominant deafness. *Nature*, **393**, 319–320.
22. Liu, X.-Z., Walsh, J., Tamagawa, Y., Kitamura, K., Nishizawa, M., Steel, K.P. and Brown, S.D.M. (1997) Autosomal dominant non-syndromic deafness caused by a mutation in the myosin VIIA gene. *Nature Genet*., **17**, 268–269.
23. Bruzzone, R., White, T.W. and Goodenough, D.A. (1996) The cellular Internet: on-line with connexins. *BioEssays*, **18**, 709–718.
24. Gyapay, G., Ginot, F., Nguyen, S., Vignal, A. and Weissenbach, J. (1996) Genotyping procedures in linkage mapping. *Methods*, **9**, 91–97.
25. Dib, C., Fauré, S., Fizames, C., Samson, D., Drouot, N., Vignal, A., Millasseau, P., Marc, S., Hazan, J., Seboun, E., Lathrop, M., Gyapay, G., Morissette, J. and Weissenbach, J. (1996) A comprehensive genetic map of the human genome based on 5,264 microsatellites. *Nature*, **380**, 152–154.