A novel biallelic splice site mutation of TECTA causes moderate to severe hearing impairment in an Algerian family

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A novel biallelic splice site mutation of *TECTA* causes moderate to severe hearing impairment in an Algerian family

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Abstract

Congenital deafness is certainly one of the most common monogenic diseases in humans, but it is also one of the most genetically heterogeneous, which makes molecular diagnosis challenging in most cases. Whole-exome sequencing in two out of three Algerian siblings affected by recessively-inherited, moderate to severe sensorineural deafness allowed us to identify a novel splice donor site mutation (c.5272+1G>A) in the gene encoding α-tectorin, a major component of the cochlear tectorial membrane. The mutation was present at the homozygous state in the three affected siblings, and at the heterozygous state in their unaffected, consanguineous parents. To our knowledge, this is the first reported *TECTA* mutation leading to the DFNB21 form of hearing impairment among Maghrebian individuals suffering from congenital hearing impairment, which further illustrates the diversity of the genes involved in congenital deafness in the Maghreb.

Introduction

Deafness is the most common congenital sensory defect. In developed countries, it is diagnosed in 1 out of 500 newborns (1-3). In about 70% of the cases, deafness is nonsyndromic, which means isolated (4). Congenital deafness is almost exclusively inherited as a monogenic disease, but a great variety of genes can be involved. It has been estimated that about 80% of the genetic forms of early onset nonsyndromic deafness are autosomal recessive (DFNB) forms, and 20% are autosomal dominant (DFNA) forms (5). Sixty DFNB genes and 32 DFNA genes have been identified so far, and many more remain to be discovered (Hereditary Hearing Loss Homepage: http://hereditaryhearingloss.org/). We report the identification of a new splice site mutation in the DFNB21 gene *TECTA* (6), encoding α-tectorin, in three Algerian siblings affected by prelingual, moderate to severe sensorineural deafness.

Patients and methods

This study was approved by the local ethics committee. Informed consent for genetic testing was obtained from the adult individuals or, in the case of minor individuals, from their parents.

Patients

Sixty-seven Algerian families with at least two members affected by prelingual bilateral deafness were recruited. Affected individuals underwent clinical examination, including pure-tone audiometry, in otolaryngology centers in Algiers, Algeria. In the vast majority of cases, deaf children were born to consanguineous normal-hearing parents, therefore indicating a DFNB form of deafness. Here, we report on such a family with three siblings affected by prelingual, moderate to severe, bilateral sensorineural hearing impairment (Figure 1A). All

three affected individuals underwent pure-tone audiometry in a soundproof room, with recording of air-conduction and bone-conduction thresholds. Air-conduction pure-tone average (ACPTA) threshold in the conversational frequencies (0.5, 1, 2, and 4 kHz) was measured for each ear, and its value for the best ear was used to define the severity of deafness: mild (20 dB < ACPTA ≤ 39 dB), moderate (40 dB < ACPTA ≤ 69 dB), severe (70 dB < ACPTA ≤ 89 dB), or profound (≥ 90 dB). Temporal bone CT scan revealed no inner ear malformation. The affected siblings had begun to walk independently at the age of nine months, and did not have any balance problems. Clinical examination failed to detect additional symptoms indicating a syndromic form of deafness, and neither proteinuria nor hematuria was detected. Both parents did not show any sign of hearing impairment.

Exome sequencing and Sanger sequencing

Genomic DNA was extracted from blood samples, using the Promega Wizard Genomic DNA Purification Kit (Promega, Madison, MI, USA, Cat. # A1120). Whole-exome sequencing was performed as previously described (7) with minor modifications, using the SureSelect V5 capture kit (Agilent) and HiSeq 2000 sequencer (Illumina). Bioinformatics analysis of sequence data was based on the pipeline provided by Illumina (CASAVA 1.8), and the algorithm ELANDv2e (Malony alignment and multi-seed mismatch reducing artifact) was used for sequence alignment. To validate the *TECTA* mutation and study its segregation by the Sanger technique, specific primers were designed using Primer3 (http://bioinfo.ut.ee/primer3-0.4.0/). The sequences of the primers are available on request.

Results and discussion

Among the DFNB families without mutations identified in the promoter, non-coding and coding exons of *GJB2*, the gene most frequently involved in Mediterranean countries (8-10),

we selected a family based on the auditory phenotype of the three affected siblings (IV.1, IV.2, and IV.3), who were affected by prelingual, symmetric, moderate to severe hearing impairment, with audiograms showing down-sloping curves (Figure 1C). We did not detect mutations in *LRTOMT*, *OTOF*, and *TMC1*, three DFNB genes previously shown to be involved in Maghrebian populations (11-14), by Sanger sequencing of the coding exons in patient IV.1. We therefore carried out whole-exome sequencing on pooled genomic DNA from two affected siblings, as previously described (7). All variants present in the dBSNP132, 1000 genomes, Exome Variant Server, and HapMap databases with a prevalence greater than 0.01% were excluded. As the parents were consanguineous, we hypothesized that the causal mutations in the affected siblings were present at the homozygous state. Using these filters, we identified a previously unreported missense mutation in *ADAMTSL2*, c.2305G>A (p.Gly769Arg), which was predicted to be damaging by the MutationTaster, SIFT, and PolyPhen2 algorithms, an indel mutation, c.46_47insCTG (p.Leu16delinsProVal), in *BRI3BP*, and the c.5272+1G>A splice site mutation in *TECTA*, a gene involved both in autosomal dominant (DFNA8/12) and autosomal recessive (DFNB21) forms of deafness (6, 15) (Figure 1B). The segregation of the *ADAMTSL2* missense mutation in this family was compatible with a causal role in the hearing impairment as the mutation was present at the homozygous state in the three affected siblings and at the heterozygous state in their normal-hearing parents. However, mutations of *ADAMTSL2* cause geleophysic dysplasia characterized by short stature, abnormalities of the hands and feet, and distinct facial features (16), but none of these clinical signs was present in any of the deaf siblings, thus indicating that the *ADAMTSL2* sequence variant was a nonpathogenic rare polymorphism. We also excluded the *BRI3BP* mutation because the third affected child (IV.3) carried this mutation at the heterozygous state. By contrast, the *TECTA* mutation, predicted to suppress the splice donor site of intron 15 (Max Ent, NNsplice, Human Splicing Finder, and Genesplicer algorithms),

was present at the homozygous state in the three affected siblings and at the heterozygous state in their parents, and it was not present in 120 Algerian control individuals. We therefore conclude that this mutation, which is expected to result in abnormal splicing of the *TECTA* mRNA, is responsible for the hearing impairment in this family.

TECTA consists of 23 exons. It encodes a 2155 amino acid secreted protein, α-tectorin, which is one of the main noncollagenous proteins of the cochlear tectorial membrane (17, 18). This ribbon-like strip of extracellular matrix lies over the cochlear hair cells and is critical both for the mechanical amplification of sound stimuli by the outer hair cells and for their transmission to the inner hair cells, which are the genuine sensory cells (19, 20). Targeted deletion of *Tecta* in mice results in a detachment of the tectorial membrane from the sensory epithelium (19). The protein is composed of a nidogen (NIDO)-like domain, a large zonadhesin (ZA) region containing three trypsin inhibitor-like (TIL) cysteine-rich domains, four C8 domains (with eight conserved cysteine residues), a von Willebrand factor type C (vWFC) and four von Willebrand factor type D (vWFD) domains, and a zona pellucida (ZP) domain (21). The *TECTA* splice site mutation identified in the Algerian family is expected to result in a truncated protein of 1765 amino acids lacking the ZP domain (Figure 1D).

TECTA has been implicated both in autosomal dominant (DFNA) and autosomal recessive (DFNB) forms of deafness (6, 15). To date, 41 and 20 different *TECTA* mutations have been reported to cause DFNA8/12 and DFNB21, respectively (Table 1). To our knowledge, none of the deaf individuals carrying the *TECTA* mutations previously reported was originating from the Maghreb. All the mutations reported in DFNA8/12, except four, are missense mutations, whereas those reported in DFNB21 are mainly, but not exclusively, truncating (i.e., nonsense, frameshift, or splice site) mutations. Of note, only two splice-site mutations leading to DFNB21 (c.2941+1G>A and c.6162+3insT) have been reported so far. The DFNA8/12 missense mutations affect amino-acid residues spread across the protein, and

produce different abnormal hearing phenotypes, with either pre- or post-lingual onset, depending on the affected protein domain. By contrast, most DFNB21 mutations are associated with similar audiometric patterns of moderate-to-severe prelingual hearing impairment, as observed in this family (6, 22, 23).

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Competing interest

The authors declare that they have no competing interest.

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Figure legend

Figure 1: Clinical and molecular data in the patients harboring the splice site mutation in *TECTA*. (A) Segregation of the *TECTA* mutation in the family. The + and – signs indicate the wild-type and mutated alleles, respectively. **(B)** Partial DNA sequencing chromatograms showing the mutation (arrow). **(C)** Air-conduction audiometric curves for both ears in patients IV.2 (open diamonds) and IV.3 (open circles) at the ages of 8 and 9 years, respectively. **(D)** Schematic representations of *TECTA* and α-tectorin. * indicates the position of the mutation in intron 15. Abbreviations: NIDO, nidogen-like domain (blue); vWFC, von Willebrand factor type C domain (purple); von Willebrand factor type D domain (yellow); C8, eight conserved cysteine residues (red); TIL, trypsin inhibitor-like cysteine-rich domain (green); ZP, zona pellucida (turquoise).

Table 1: List of the *TECTA* mutations reported in DFNA8/12 and DFNB21

Inheritance pattern	*TECTA* mutation	Exon	Protein domain	Severity of deafness	Age of onset	Progression	Affected sound frequencies	Family origin	Reference
AD	c.257_262delinsGCT; p.Ser86_Pro88delinsCysSer	3		moderate	postlingual	progressive	all	Chinese	[24]
AR	c.266delT; p.Leu89Argfs*34	3	ENT	moderate to severe	prelingual	stable	mid	Iranian	[22]
AD	c.589G>A; p.Asp197Asn	4	ENT		postlingual	stable	mid	American	[25]
AR	c.596delT; p.Leu199Argfs*7	4	ENT	profound	prelingual	progressive	mid	Japanese	[26]
AD	c.632T>C; p.Phe211Ser	5	ENT		postlingual	stable	mid	Spanish	[25]
AR	c.651dup; p.Asn218Glnfs*31	5	ENT	moderate	prelingual	stable	all	Iranian	[23]
AR	c.654_657delTTTC; p.Phe219Serfs*12	5	ENT	moderate to profound	prelingual			British	[27]
AD	c.710C>T; p.Thr237Ile	5	ENT	moderate	postlingual	stable	high	Korean	[28]
AD	c.775G>C; p.Gly259Arg	5		moderate to severe	postlingual	progressive	all	Italian	[29]
AD	c.950T>A; p.Val317Glu	6	ZA		postlingual		high	Korean	[30]
AR	c.990C>A; p.Tyr330*	6	ZA (vWFD1)	severe	prelingual			Chinese	[31]
AD	c.1084A>T; p.Ser362Cys	6	ZA (vWFD1)		postlingual		mid	American	[25]
AD	c.1124delT; p.Val375Alafs*4	6	ZA (vWFD1)		postlingual		mid	Spanish	[25]
AD	c.1395T>G; p.Asn465Lys	7	ZA (vWFD1)		postlingual	progressive	mid	Belgian	[25]
AR	c.1471C>T; p.Arg491Cys	7	ZA (vWFD1)	profound	prelingual	progressive	mid	Japanese	[26]
AD	c.1685C>T; p.Thr562Met	7	ZA		postlingual		mid	American	[25]
AR	c.2428C>T; p.Arg810*	9	ZA (vWFD2)		prelingual			British	[32]
AD	c.2444C>T; p.Thr815Met[a]	9	ZA (vWFD2)		prelingual		mid	American	[25]
AR	c.2592C>A; p.Asn864Lys	9	ZA	moderate to	prelingual			British	[27]

			(vWFD2)	profound					
AD	c.2657A>G; p.Asn886Ser[a]	9	ZA (vWFD2)		prelingual	progressive	high	British	[25]
AR	c.2941+1G>A	intron 9	ZA	severe to profound	prelingual		all	Lebanese	[33]
AD	c.3107G>A; p.Cys1036Tyr	10	ZA (TIL2)		postlingual	stable	mid	Spanish	[25]
AR	c.3123G>C; p.Glu1041Asp	10	ZA		prelingual			British	[32]
AD	c.3169T>A; p.Cys1057Ser	10	ZA	mild to severe	postlingual	progressive	high	Swedish	[34]
AD	c.3293C>T; pAla1098Val	10	ZA		postlingual		high	Spanish	[25]
AD	c.3406G>C; p.Asp1136His[a]	10	ZA (vWFD3)		postlingual		high	Spanish	[25]
AD	c.3743C>T; p.Pro1248Leu[a]	11	ZA (vWFD3)		prelingual		high	Spanish	[25]
AR	c.3903C>A; p.Cys1301*	11	ZA (vWFD3)	moderate	prelingual	stable		Iranian	[35]
AR	c.4055G>A; p.Cys1352Tyr	11	ZA	moderate to profound	prelingual			British	[27]
AD	c.4198C>T; p.His1400Tyr	12	ZA (TIL3)		postlingual			Japanese	[36]
AD	c.4525T>G; p.Cys1509Gly	13	ZA (vWFD4)			progressive	high	Turkish	[37]
AD	c.4549T>C; p.Cys1517Arg	13	ZA (vWFD4)		postlingual		high	Spanish	[25]
AD	c.4856G>C; p.Cys1619Ser	14	ZA (vWFD4)	mild to moderate-severe	variable	progressive	high	French	[38]
AR	c.4857C>A; p.Cys1619*	14	ZA (vWFD4)	profound	prelingual			Palestinian	[39]
AR	c.5072G>T; p.Cys1691Phe	15	ZA (vWFD4)	moderate to severe	prelingual	stable		Korean	[40]
AR	c.5211C>A; p.Tyr1737*	15	ZA	moderate to severe	prelingual	stable	mid	Iranian	[22]
AR	c.5210A>G; p.Tyr1737Cys	15	ZA		prelingual			Iranian	[41]
AR	**c.5272+1G>A**	**intron 15**	**ZA**	**moderate to severe**	**prelingual**	**stable**	**all**	**Algerian**	**This study**
AR	c.5317C>T; p.Arg1773*	16	ZA					Japanese	[42]

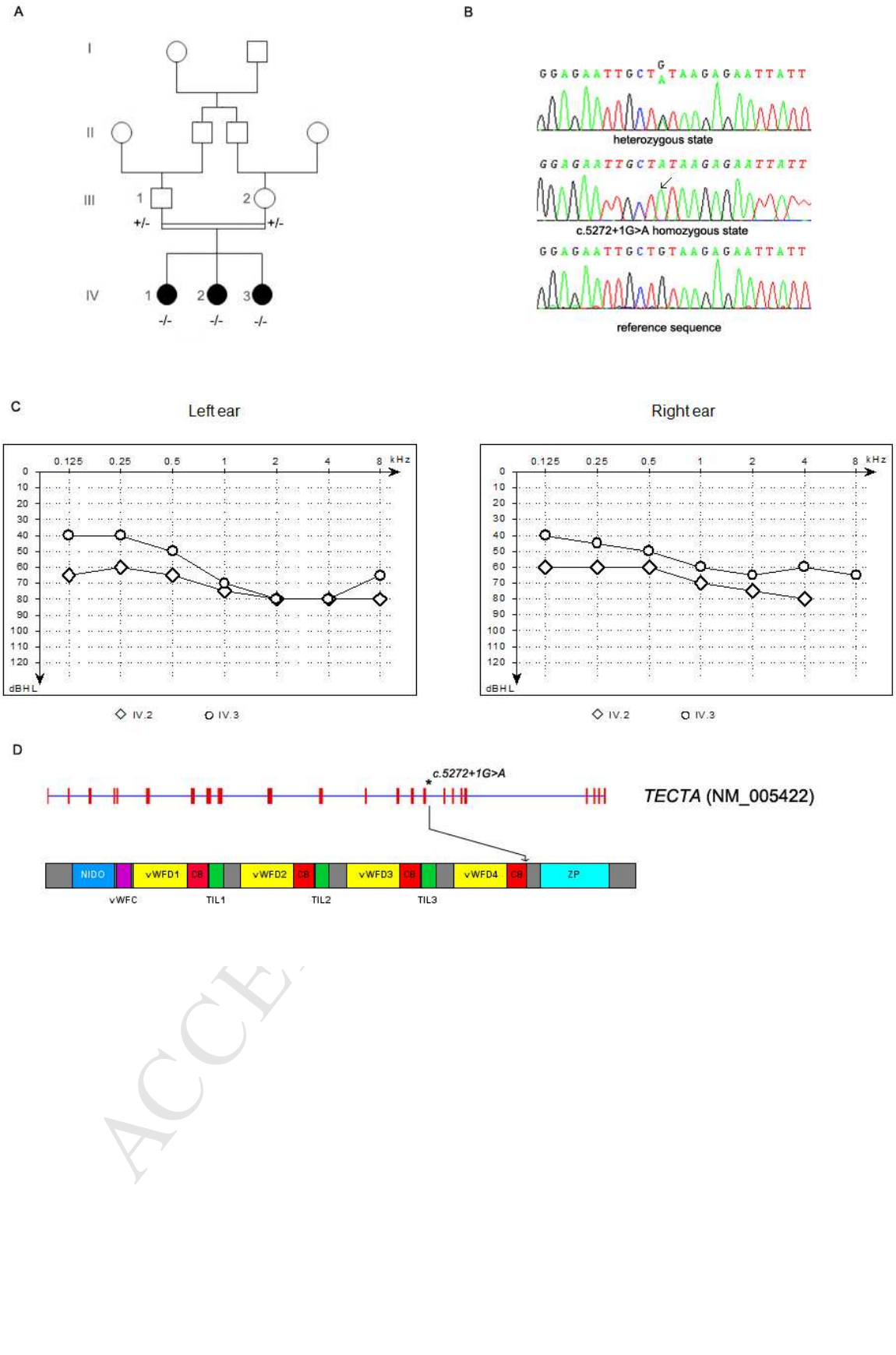
AD	c.5331G>A; p.Leu1777Leu	16	ZA		prelingual	stable	mid	Dutch	[43]
AD	c.5372C>G; p.Pro1791Arg	16	ZA		prelingual		high	American	[25]
AD	c.5383+2T>G	intron 16	ZA		prelingual	stable	mid	Spanish	[25]
AD	c.5383+5delGTGA	intron 16	ZA		prelingual		high	British	[25]
AD	c.5458C>T; p.Leu1820Phe	17	ZP	mild to severe	postlingual	stable	mid	Belgian	[44]
AD	c.5471G>A; p.Gly1824Asp	17	ZP	mild to severe	postlingual	stable	mid	Belgian	[44]
AD	c.5509T>G; p.Cys1837Gly	17	ZP	mild to severe	postlingual	progressive	mid	Spanish	[25, 45]
AD	c.5509T>C; p.Cys1837Arg	17	ZP	mild to moderate	postlingual	progressive	mid	American	[22]
AD	c.5597C>T; p.Thr1866Met	18	ZP	mild to moderate	postlingual	stable/ progressive	mid	Korean/Spanish/ American	[25, 30]
AD	c.5600A>G; p.His1867Arg	18	ZP		postlingual	progressive	mid	Spanish	[25]
AD	c.5609A>G; p.Tyr1870Cys	18	ZP	moderate to severe	prelingual	stable	mid	Austrian	[44]
AD	c.5618 C>T; p.Thr1873Ile	18	ZP	moderate		stable	mid	Korean	[46]
AD	c.5668C>T; p.Arg1890Cys	18	ZP	mild to moderate	prelingual	stable	mid	Dutch/Spanish/ American	[25, 47]
AD	c.5692T>C; p.Cys1898Arg	18	ZP		postlingual		mid	American	[25]
AD	c.5839C>T; p.Arg1947Cys	19	ZP		postlingual		mid	American	[25]
AD	c.5945C>A; p.Ala1982Asp	19	ZP	moderate to severe	prelingual	progressive	all	Chinese	[48]
AD	c.5990T>C; p.Ile1997Thr	19	ZP		prelingual	progressive	mid	Japanese	[36]
AD	c.6016G>T; p.Asp2006Tyr	20	ZP	severe	prelingual		mid	Mongolian	[49]
AD	c.6026T>C; p.Ile2009Thr	20	ZP		postlingual	stable	high	Spanish	[25]
AD	c.6062G>A; p.Arg2021His	20	ZP	mild to moderate	prelingual	stable	mid	Japanese	[50]
AR	c.6037delG; p.Glu2013Argfs*6	20	ZP	moderate to severe	prelingual	stable	mid (flat to shallow U-shaped audiogram)	Pakistani	[23]
AR	c.6162+3insT	intron 20	ZP	moderate to severe	prelingual	stable		Korean	[40]

AR	c.6203_6218del; p.Lys2068Argfs*38	21	ZP	moderate	prelingual	stable	all (flat to shallow U shaped audiogram)	Iranian	[51]

AD, autosomal dominant (DFNA8/12)

AR, autosomal recessive (DFNB21)

[a] Pathogenicity questionable


A
I
II
III
1
2
+/-
+/-
IV
1
2
3
-/-
-/-
-/-
B
heterozygous state
c.5272+1G>A homozygous state
reference sequence
C
Left ear
Right ear
0.125
0.25
0.5
1
2
4
8
kHz
dBHL
IV.2
IV.3
D
c.5272+1G>A
TECTA (NM_005422)
NIDO
vWFC
vWFD1
CB
TIL1
vWFD2
CB
TIL2
vWFD3
CB
TIL3
vWFD4
CB
ZP