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Mapping of the α-Tectorin Gene (*TECTA*) to Mouse Chromosome 9 and Human Chromosome 11: A Candidate for Human Autosomal Dominant Nonsyndromic Deafness

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α-Tectorin is one of the major noncollagenous components of the mammalian tectorial membrane in the inner ear. We have mapped the gene encoding α-tectorin to mouse chromosome 9 and human chromosome 11 in a known region of conserved synteny. Human YAC clones containing α-tectorin have been identified, demonstrating physical linkage to the anonymous marker *D11S925*. This places α-tectorin within the genetic interval that contains both the human nonsyndromic autosomal dominant deafness DFNA12 and the proximal limit of a subset of deletions within Jacobsen syndrome. Thus both DFNA12 and the hearing loss in some cases of Jacobsen syndrome may be due to haploinsufficiency for *TECTA*.

INTRODUCTION

The tectorial membrane plays a crucial role in the transduction of mechanical energy into neural potentials in the cochlea. Shear generated between the lower surface of the tectorial membrane and the apical surface of the auditory epithelium as a result of sound-induced motion of the basilar membrane is considered the primary stimulus for hair cells. The mammalian tectorial membrane is a sheet of extracellular matrix composed of both collagenase-sensitive (collagens Type II, V, and IX) and -insensitive proteins (Richardson *et al.*, 1987; Thalmann *et al.*, 1987). These noncollagenous glycoproteins may account for up to 50% of the total protein of the tectorial membrane (Richardson *et al.*, 1987) and have accordingly been termed tectorins (Killick *et al.*, 1995). SDS–PAGE under reducing conditions resolves the mouse tectorins into high, medium, and low molecular weight fractions of 173, 60, and 45 kDa (Killick and Richardson, 1997). These fractions are derived from a single large protein, α-tectorin, with a smaller protein, β-tectorin, also contributing to the low molecular mass fraction (Legan *et al.*, 1997). The tectorins are encoded by two single-copy genes in the mouse, *α-tectorin* (*Tecta*) and *β-tectorin* (*Tectb*) (Legan *et al.*, 1997). Both proteins share similarities with components of the sperm–egg adhesion system (Legan *et al.*, 1997).

As α-tectorin is a significant component of the tectorial membrane, mutations in the *Tecta*/*TECTA* genes may be involved in deafness in either mouse (Steel, 1995) or human (Van Camp *et al.*, 1997). It is already known that mutations in collagen genes can cause abnormalities of the inner ear and deafness (Hughes, 1997). To ascertain any involvement of α-tectorin in hearing impairment first requires that the chromosomal location of the gene be determined in either human or mouse. We now report the chromosomal locations of *Tecta* in mouse and *TECTA* in human.

MATERIALS AND METHODS

Materials. Genomic DNA from parental mouse strains (C57BL/6J and *Mus spretus*) and the European Collaborative Interspecific Backcross (EUCIB), from the human–rodent monochromosomal somatic cell hybrid panel, and from human YAC clones were obtained from the Human Genome Mapping Resource Centre (Hinxton, UK). Primers for human chromosome 11 markers were obtained from Research Genetics.

Methods. PCR was performed with AmpliTaq (Applied Biosystems) according to the manufacturer's instructions. PCR primers used were as follows: 83GF4, 5′-CAGGATCGCACAGATTATTC-3′; 83GR18, 5′-AATTAGAGGCTCTGCAGCTC-3′; 83GF2, 5′-CCAATGGCACTCATATCATG-3′; and 83GR17, 5′-GAGATCTTGATATGTAGCTC-3′. The conditions for PCR were 40 cycles of 95°C for 10 s, 55°C (83GF4/GR18) or 51°C (83GF2/GR17) for 5 s, and 72°C for 60 s. PCR was performed on 50–100 ng of genomic DNA. Yeast colonies were lysed by heating to 95°C in 100 μl of TE; 1 μl of this lysate was then used for PCR. For screening the human–rodent monochromosomal somatic cell hybrid panel, 5 μl of PCR product was placed in a final reaction volume of 50 μl of 1× One-Phor-All Buffer and digested with *Taq*I (Pharmacia) at 65°C for 1 h. Samples were electrophoresed on 3% agarose gels in 0.5× TBE.

Mapping data from this article have been deposited with the Mouse Genome Database under Accession No. MGD-JNUM-43211 and with the Genome Data Base under Accession No. 9255418.

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RESULTS

Mapping of Mouse Tecta

We decided to determine the chromosomal location of the murine *Tecta* gene first. The EUCIB represents a useful resource for the mapping of mouse loci (Breen *et al.*, 1994). The only requirement for mapping on EUCIB is to be able to differentiate between the alleles of a particular locus for C57BL/6J and *M. spretus*. A selection of primer pairs based on the mouse *Tecta* cDNA sequence (Legan *et al.*, 1997) was assessed in PCR analysis for amplification of, and polymorphism between, C57BL/6J and *M. spretus* DNA. Primer pair 83GF4 and 83GR18, which amplifies between adjacent exons in the murine *Tecta* gene, generates a slightly smaller product in C57BL/6J compared to *M. spretus* (approx 500 bp). Subsequently, 51 samples from EUCIB were genotyped for this polymorphism (data available at http://www.hgmp.mrc.ac.uk/Mbx/MBxHomepage.html), enabling the localization of *Tecta* to mouse chromosome 9; the locus order was determined to be *D9Mit227, D9Mit328, D9Mit254–D9Mit329, D9Mit140–Tecta–D9Nds10–D9Mit255, D9Mit23* (Fig. 1a).

Mapping of the Human TECTA Gene

According to Debry and Seldin (1996), this region of mouse chromosome 9 shows conserved synteny with human chromosome 11. BLAST searches of sequence databases identified two human IMAGE clones with significant sequence homology to the mouse *Tecta* gene (IMAGE ID 180068, Accession Nos. R84585 and R85474; IMAGE ID 112850, Accession Nos. T86051 and T86996). Additional sequencing of both clones has confirmed that these correspond to the human *TECTA* gene; the homology between the mouse and human sequence is 88% in the coding region and 70% in the 3′ untranslated region (D.C.H., unpublished data). Comparison of the mouse and human sequences identified a *Taq*I site present in the human sequence and not in the mouse sequence (R84585; nucleotide 184). Human, mouse, and hamster genomic DNA were amplified with primer pair 83GF2 and 83GR17, giving similar-sized products (Fig. 2a). The PCR products were then digested with *Taq*I, demonstrating the polymorphism (Fig. 2a); thus it was possible to utilize a human monochromosomal somatic cell hybrid panel wherein individual human chromosomes have been retained on a rodent (mouse or hamster) background (Kelsell *et al.*, 1995). DNA samples from the panel were typed for the polymorphism. Human-specific *Taq*I fragments were present in hybrids containing human chromosomes 11 and 15 (Fig. 2b). The human chromosome 11 locus most likely corresponds to that mapped to mouse chromosome 9 based on comparison of flanking loci (Debry and Seldin, 1995). As the *Taq*I polymorphism is shared between the chromosome 11 and 15 loci, it is probable that either these loci represent a recent duplication or the chromosome 15 somatic cell hybrid is contaminated with a fragment from human chromosome 11. Southern analysis of genomic DNA has suggested the presence of only a single *Tecta* gene in the mouse (Legan *et al.*, 1997).

Physical Localization of the Human TECTA Gene

To localize the human *TECTA* gene further on chromosome 11, we examined more closely the region of conserved synteny between human chromosome 11 and mouse chromosome 9 (Debry and Seldin, 1996; Hudson *et al.*, 1995). A selection of human YAC clones was identified from data at The Whitehead Institute/MIT Center for Genomic Research (Hudson *et al.*, 1995), covering the interval between *D11S4090* and *D11S925* (YAC clone IDs 928F11, 957E4, 771D4, 939B12, 911F2, 822G8, 785C6, 742F9, 969D7, and 936D9). Lysates from these YAC clones were amplified with primer pair 83GF2/83GR17; a positive PCR result was noted for 936D9 (Table 1), placing *TECTA* in the vicinity of *D11S925*. Additional YACS containing *D11S925* and/or *NIB947* were tested for *TECTA* (Table 1). On the basis of these results TECTA is likely to be distal of *D11S925*, but the order with respect to *NIB947* cannot be determined from the available data (Fig. 1b).

Confirmation of TECTA as a Single-Copy Gene

The analysis of the human monochromosomal somatic cell hybrid panel suggested the existence of two highly homologous *TECTA* loci in the human genome, whereas in contrast there is only a single locus in the mouse (Legan *et al.*, 1997). An alternative explanation is that the human chromosome 15 monochromosomal somatic cell hybrid contains an additional fragment of chromosome 11 that includes the *TECTA* gene. To test this hypothesis, a subset of the monochromosomal somatic cell hybrid panel, including the chromosome 11 and 15 hybrids, was tested for the presence of a marker linked to *TECTA* (*D11S925*) and three proximal flanking markers (*D11S908*, *923*, and *927*). Both the chromosome 11 and 15 hybrids were positive for *D11S925* (data not shown), but *D11S908*, *923*, and *927* were only present in the chromosome 11 hybrid. Thus it is most likely that the chromosome 15 hybrid is contaminated with a fragment from human chromosome 11 that includes *TECTA* and *D11S925*, the proximal breakpoint of which lies between *D11S908* and *D11S925*. As such we can state that like the mouse, the human genome only contains a single *TECTA* gene.

DISCUSSION

In recent years there has been a dramatic increase in the localization of genes for autosomal dominant and autosomal recessive nonsyndromic hearing impairment (Van Camp *et al.*, 1997). However, in many cases the number of families segregating any one locus is small, adding to the difficulty in identifying the gene responsible (Van Camp *et al.*, 1997). The mouse pro-

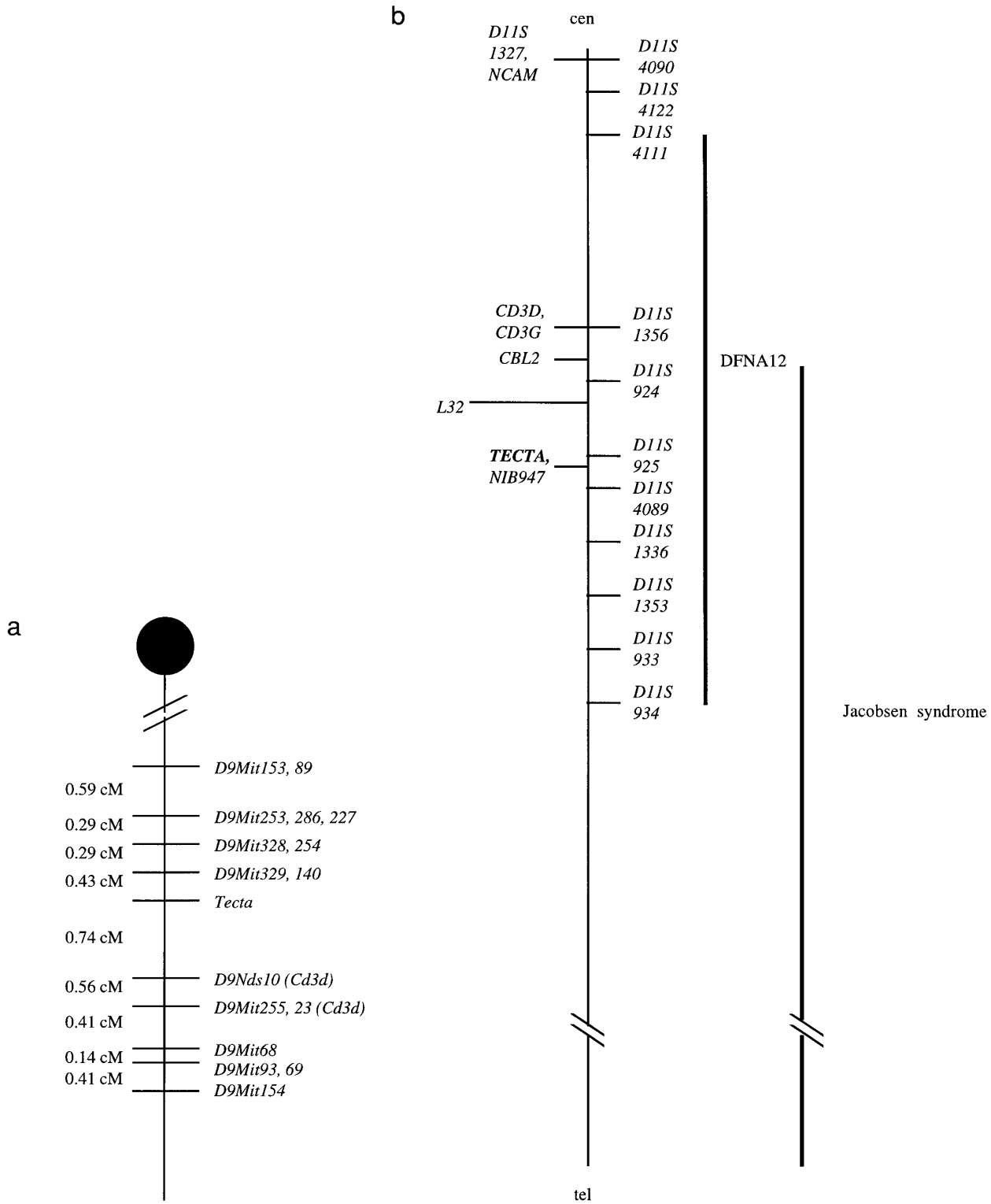

FIG. 1. (**a**) Genetic location of mouse *Tecta* gene. The segregation of *Tecta* on EUCIB enabled the gene to be localized to chromosome 9; marker order and genetic distances were determined using the MBx program. (**b**) Location of the human *TECTA* gene. The locations of *TECTA*, DFNA12, and the proximal limit of the breakpoints in Jacobsen syndrome are indicated, derived from data in Hudson *et al.* (1995), Penny *et al.* (1995), Verhoeven *et al.* (1997), and herein.

vides a model for many human developmental disorders, including deafness (Steel, 1995). Similarly, the existence of high-resolution mouse genetic maps allows the mapping of mouse genes with relative ease (Breen *et al.*, 1994; Dietrich *et al.*, 1996), from which, on the basis of the conservation of synteny, the location of the human gene can be inferred or confirmed (Debry and Seldin, 1996). We have determined the location of the

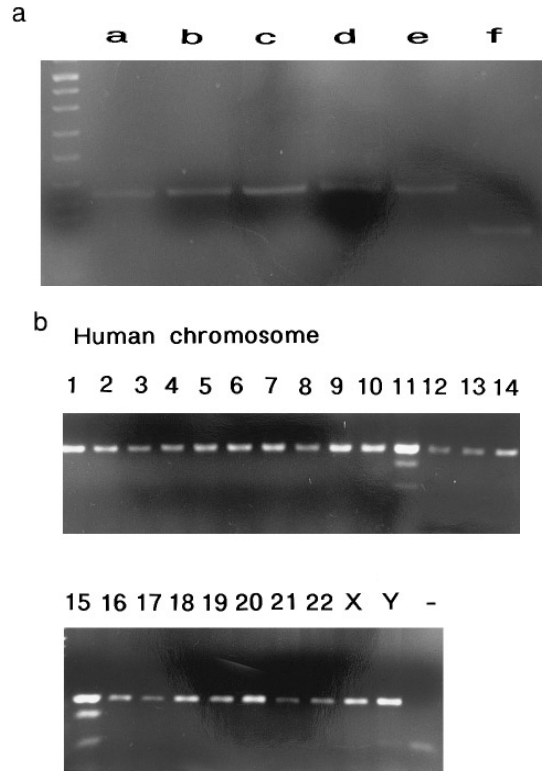

FIG. 2. Chromosomal localization of human *TECTA* gene. (**a**) Demonstration of a human-specific polymorphism. Genomic DNA was amplified with 83GF2/83GR17 and electrophoresed on a 3% agarose gel. Undigested PCR products lanes **a,** mouse; **b,** hamster; **c,** human; PCR products digested with *Taq*I lanes **d,** mouse; **e,** hamster; **f,** human. Markers are marker VIII (Boehringer Mannheim). (**b**) Analysis of the human monochromosomal somatic cell hybrid panel (Kelsell *et al.*, 1995). DNA from each hybrid was amplified with 83GF2/83GR17, digested with *Taq*I, and electrophoresed on a 3% agarose gel.

gene encoding α-tectorin, a major component of the tectorial membrane in the inner ear, to mouse chromosome 9 and human chromosome 11, in a region of known conservation of synteny (Debry and Seldin, 1996). Analysis of a human monochromosomal somatic cell hybrid panel (Kelsell *et al.*, 1995) suggested the presence of two highly similar human *TECTA* genes; however, subsequent analysis identified a previously undescribed contamination of the chromosome 15 hybrid with a fragment derived from chromosome 11, the proximal limit of which lies between *D11S908* and *D11S925*. The distal limit of this fragment has not yet been determined. Whether this is a common problem with this monochromosomal somatic cell hybrid panel is not known, but this may lead to confusion when attempting to determine the chromosomal location of human genes. No mouse mutations that cause deafness are known to localize to the region of mouse chromosome 9 to which we have mapped α-tectorin (Steel, 1995). However, a number of interesting human loci have been mapped previously to the conserved region of chromosome 11, including at least one putative tumor suppressor gene (Davis *et al.*, 1996; Gabra *et al.*, 1996). More significantly, the human autosomal dominant nonsyndromic deafness DFNA12 has been mapped to a 36-cM interval on 11q22–q24, between *D11S4120* and *D11S912* (Verhoeven *et al.*, 1997). Affected individuals display mild to moderately severe bilateral sensorineural hearing loss, mainly in the middle frequencies (500–2000 Hz) with a prelingual onset (Verhoeven *et al.*, 1997). *D11S925* maps in the middle of this region, cosegregating with DFNA12 in this pedigree (Verhoeven *et al.*, 1997). As we have demonstrated, *D11S925* and *TECTA* are physically linked, so *TECTA* represents a very good candidate for the DFNA12 locus. There is preliminary evidence that another locus for autosomal dominant nonsyndromic hearing impairment (DFNA8) also maps to 11q (Van Camp *et al.*, 1997); this may represent another allele of DFNA12. Thus *TECTA* may also be considered a candidate for DFNA8. It is interesting to note that α-tectorin is expressed only transiently during mouse cochlear development (A. Rau, P. K. Legan, and G. P. Richardson,

TABLE 1

Physical Localization of *TECTA*

YAC	*WI-8803*	*D11S925*	*D11S4107*	*D11S4089*	*TECTA*	*NIB947*	*FB17D4*
936D9	+	+	+	+	+	+	–
969D7	+	–	–	–	–	–	–
795G11	–	+	–	+	–	–	–
758F8	–	+	–	+	–	–	–
788B9	–	–	–	+	+	+	+
806B12	–	–	–	–	–	+	–
908D11	–	–	–	+	+	+	–
800A10	–	–	–	–	–	+	–
857E6	–	–	–	–	–	+	–
921G9	–	–	–	–	+	+	–
926E4	–	–	+	–	+	+	–
946G6	–	–	–	–	+	+	–

Note. Localization of *TECTA* on YACs containing fragments of human chromosome 11. The markers are arranged in the probable order that they are in on chromosome 11. Data for all markers apart from *TECTA* were obtained from The Whitehead Institute/MIT Center for Genome Research (Hudson *et al.*, 1995).

unpublished observations), and both DFNA12 and DFNA8 are prelingual in onset and stable (Van Camp *et al.*, 1997).

The nature of the DFNA12 mutation may be clarified by the findings on a human syndromic disorder. Jacobsen syndrome, first described in 1973 (Jacobsen *et al.*, 1973), is caused by segmental aneusomy for the distal end of the long arm of chromosome 11 (Lewanda *et al.*, 1995; Penny *et al.*, 1995). The typical features, though not always present, include mild to moderate pyschomotor retardation, trigoncephaly, facial dysmorphism, cardiac defects, and thromobocytopenia; thus it is appropriate to consider Jacobsen syndrome a contiguous gene disorder. Hearing loss or abnormality has been described as a characteristic feature of Jacobsen syndrome by Lee and Sciorra (1981) (see also Linarelli *et al.*, 1975 and Pivnick *et al.*, 1996), although the overall frequency is not clear (Lewanda *et al.*, 1995; Penny *et al.*, 1995). Characterization of the chromosomal breakpoints in Jacobsen patients has delineated the various abnormalities associated with Jacobsen syndrome (Penny *et al.*, 1995). It has been demonstrated that some cases of Jacobsen syndrome are associated with the expression of a fragile site (*FRA11B*), which has been shown to correspond to an expanded repeat in the *CBL2* gene (Jones *et al.*, 1994, 1995). Penny *et al.* (1995) have extended the molecular characterization of 11q deletions in Jacobsen patients and have identified *D11S925* as one of the most proximal markers to be deleted; based on our mapping, *TECTA* is distal of *CBL2* (Fig. 1b).

We can draw several conclusions based on the location of the human *TECTA* gene. It maps to the interval defined as containing DFNA12 and represents a highly plausible candidate for this human deafness. It is highly likely to be deleted in a subset of 11q− in Jacobsen syndrome and may be responsible for the previously reported hearing loss as a consequence of haploinsufficiency. If so, then DFNA12 may also be due to haploinsufficiency for *TECTA*. A 50% reduction in the amount of TECTA protein may also affect the levels of TECTB, the other major noncollagenous component of the tectorial membrane. Similar phenomena have been reported in other extracellular matrices; levels of ZP2 protein in the zona pellucida of the egg drop to 50% of normal in mice heterozygous for a null allele of ZP3 (Wassarman *et al.*, 1997), and there is evidence for coordinate expression of the α-3, -4, and -5 chains of collagen Type IV (Thorner *et al.*, 1996; Kalluri *et al.*, 1997). Alternatively, DFNA12 may be due to gain of function mutation in α-tectorin. The relationship between *TECTA* and DFNA12 is currently under investigation.

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