

Mutations in *TBC1D24*, a Gene Associated With Epilepsy, Also Cause Nonsyndromic Deafness DFNB86

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Inherited deafness is clinically and genetically heterogeneous. We recently mapped *DFNB86*, a locus associated with nonsyndromic deafness, to chromosome 16p. In this study, whole-exome sequencing was performed with genomic DNA from affected individuals from three large consanguineous families in which markers linked to *DFNB86* segregate with profound deafness. Analyses of these data revealed homozygous mutation c.208G>T (p.Asp70Tyr) or c.878G>C (p.Arg293Pro) in *TBC1D24* as the underlying cause of deafness in the three families. Sanger sequence analysis of *TBC1D24* in an additional large family in which deafness segregates with *DFNB86* identified the c.208G>T (p.Asp70Tyr) substitution. These mutations affect *TBC1D24* amino acid residues that are conserved in orthologs ranging from fruit fly to human. Neither variant was observed in databases of single-nucleotide variants or in 634 chromosomes from ethnically matched control subjects. *TBC1D24* in the mouse inner ear was immunolocalized predominantly to spiral ganglion neurons, indicating that *DFNB86* deafness might be an auditory neuropathy spectrum disorder. Previously, six recessive mutations in *TBC1D24* were reported to cause seizures (hearing loss was not reported) ranging in severity from epilepsy with otherwise normal development to epileptic encephalopathy resulting in childhood death. Two of our four families in which deafness segregates with mutant alleles of *TBC1D24* were available for neurological examination. Cosegregation of epilepsy and deafness was not observed in these two families. Although the causal relationship between genotype and phenotype is not presently understood, our findings, combined with published data, indicate that recessive alleles of *TBC1D24* can cause either epilepsy or nonsyndromic deafness.

Hearing loss occurs in approximately 0.2% of newborns, and two-thirds of these cases appear to have a genetic cause.¹ The prevalence of hearing loss increases with age, and there are hundreds of syndromes that include deafness as one feature of a complex phenotype (OMIM, see [Web Resources](#)). Additionally, 114 loci have been genetically mapped for nonsyndromic deafness segregating as either a dominant (DFNA) or a recessive (DFNB) trait (Hereditary Hearing Loss Homepage, see [Web Resources](#)).^{1,2} For approximately half of these loci, the genes with mutations that result in nonsyndromic deafness have been identified. The wild-type alleles of these genes subserve a myriad of cellular functions that span the gamut from transcription factors to extracellular matrix proteins,^{2,3} and one-third of them encode proteins that interact with actin and are crucial for mechanotransduction of sound in the inner ear.^{4,5} Despite unique anatomical structures and physio-

logical functions described in the auditory system,^{6,7} there are only a few examples of genes that have a pattern of expression limited to the inner ear.^{8,9} In fact, many genes with mutations associated with nonsyndromic deafness do not encode inner-ear-cell-specific molecules but rather are expressed in a variety of organ systems.^{10–12} For example, *ACTG1* (MIM 102560), encoding cytoplasmic γ -actin,¹³ and *HGF* (MIM 142409), encoding hepatocyte growth factor (HGF), are associated with nonsyndromic deafness DFNA20 (MIM 604717) and DFNB39 (MIM 608265), respectively, and show widespread expression.¹⁴ An inference from these observations is that a mutation in any gene, no matter how widely expressed in the body, could be a cause of a phenotypically restricted human disorder.^{15,16}

Genome-wide homozygosity mapping of DFNB in a large consanguineous Pakistani family (PKDF799) identified the

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<http://dx.doi.org/10.1016/j.ajhg.2013.12.004>. ©2014 by The American Society of Human Genetics. All rights reserved.

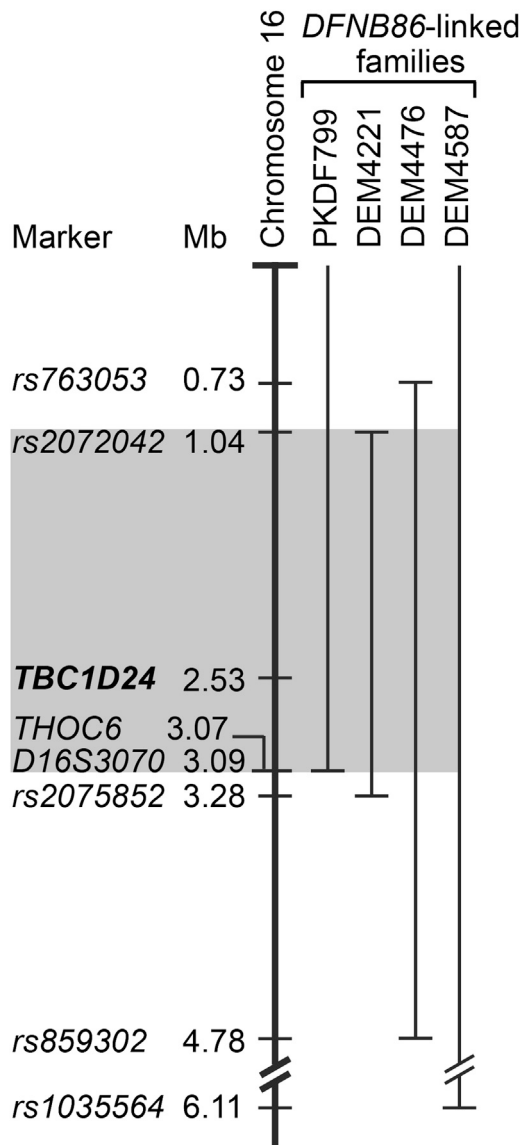


Figure 1. Refinement of the *DFNB86* Linkage Interval

The thick vertical bar represents human chromosome 16p. The linkage interval for the deafness segregating in each of the four families is indicated by a thin vertical bar. The gray shaded region highlights the *DFNB86* linkage interval shared by the four families.

locus *DFNB86* on chromosome 16p with a maximum LOD score of 8.5.¹⁷ In this study, three additional consanguineous pedigrees (DEM4221, DEM4587, and DEM4476) from Pakistan were ascertained (inbreeding coefficients, Table S1, available online), and their recessive deafness was linked to *DFNB86* with maximum LOD scores of 6.90, 3.26, and 5.97, respectively. The overlapping region of homozygosity of these four pedigrees spans 2.05 Mb and contains 121 annotated genes (Figure 1). Here, we report that recessive mutations in *TBC1D24* (TBC1 domain family, member 24 [MIM 613577]) are the cause of the nonsyndromic deafness (*DFNB86*) segregating in these four families.

Approval for this study was obtained from the institutional review boards of the Baylor College of Medicine

and Affiliated Hospitals (Houston), the National Centre of Excellence in Molecular Biology (University of the Punjab, Lahore), and Quaid-I-Azam University (Islamabad) and from the Combined Neuroscience Institutional Review Board (protocol OH93-DC-0016) at the National Institutes of Health (Bethesda). Written informed consent was obtained from all family members participating in this study.

Pure-tone audiometric evaluations of 4 of the 11 affected individuals from family PKDF799 revealed profound deafness (hearing threshold ≥ 90 dB) at all test frequencies, whereas obligate carriers had normal hearing thresholds.¹⁷ Genomic DNA samples from three affected individuals (IV-23 from family PKDF799, IV-11 from family DEM4221, and IV-6 from family DEM4476) were processed for whole-exome sequencing (WES; Figure 2). For family PKDF799, a TargetSeq Exome Enrichment Kit (Applied Biosystems) was used for capturing the whole exome (45.1 Mb), which was sequenced on an Applied Biosystems SOLiD5500 platform. For families DEM4221 and DEM4476, an EZ Exome v.3.0 kit (NimbleGen) was used for capturing ~ 64 Mb of protein-coding plus untranslated expressed sequences, and massively parallel sequencing was performed on an Illumina HiSeq. Sequence reads generated from these libraries were filtered for quality and were mapped to the hg19 human reference genome (UCSC Genome Browser). For family PKDF799, mapping and variant calling were performed with LifeScope (Applied Biosystems). ANNOVAR was used for variant analysis.¹⁸ For families DEM4221 and DEM4476, sequence alignment and variant calling were performed with the Burrows-Wheeler Aligner and Genome Analysis Toolkit, respectively. Depth of coverage and the number of DNA variants in the three WES data sets are summarized in Table S2. Assuming locus homogeneity, and because there is significant evidence of linkage between markers for *DFNB86* and the deafness segregating in these families, we focused our evaluation of the WES data sets only on DNA variants in the smallest *DFNB86* linkage interval defined by meiotic recombinations (Figure 1). Additional criteria for filtering data for identifying the pathogenic variants were homozygosity for a variant and an allele frequency $< 1\%$ in the NHLBI Exome Sequencing Project (ESP) Exome Variant Server (EVS)¹⁹ and the 1000 Genomes Project. These variants were confirmed by Sanger sequence analysis of genomic DNA from family members, and allele frequencies were obtained with the use of ethnically matched control individuals (≥ 148 chromosomes, Table 1).

On the basis of the criteria listed above, analyses of the WES data sets for the three families revealed a total of seven homozygous DNA variants within the refined *DFNB86* interval (Table 1). Five of the seven variants were not considered to be pathogenic. A synonymous variant, c.3060G>A (p.Ala1020Ala), in *CACNA1H* (MIM 607904) is a polymorphism in the Pakistani population. The c.1520G>A (p.Arg507His) variant in *IGFALS* (MIM 601489) was not studied further because the affected

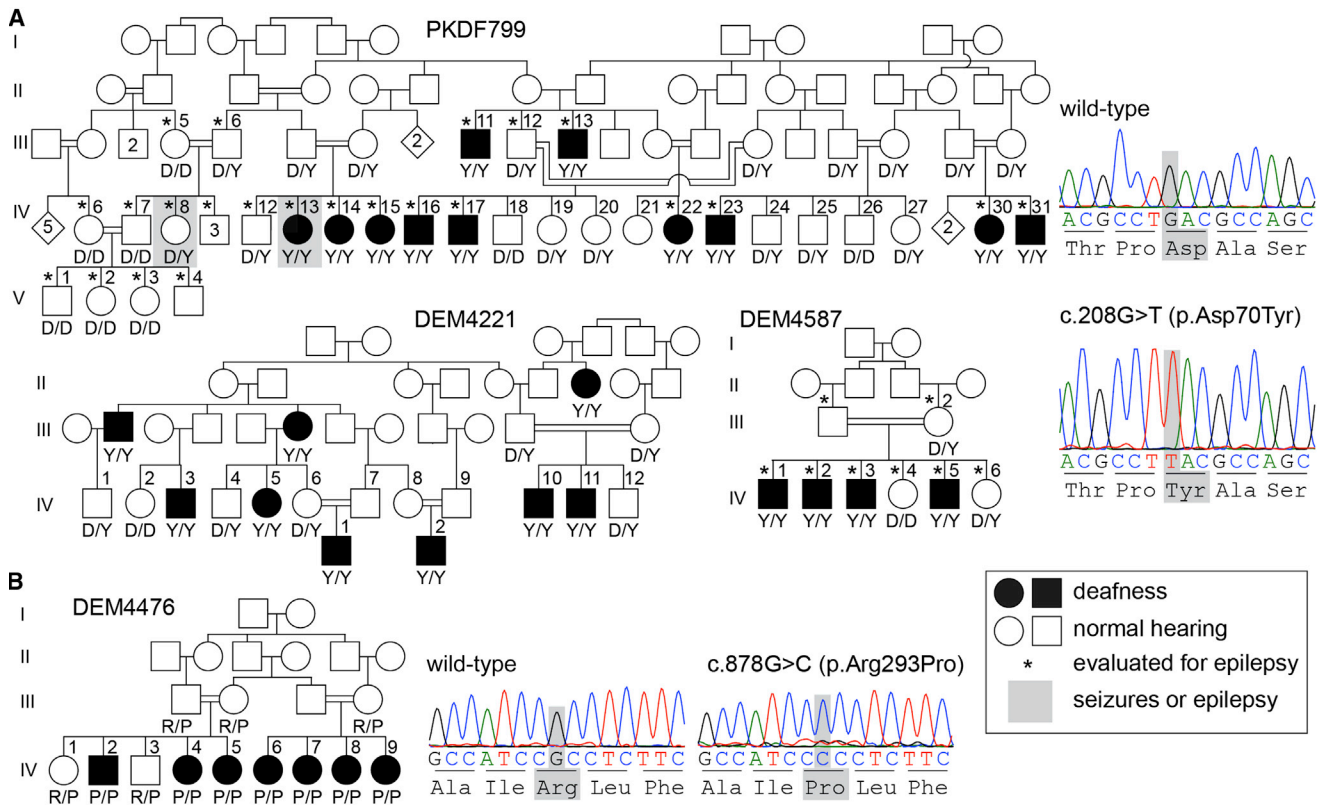


Figure 2. Four Families in which Deafness Cosegregates with Missense Mutations in *TBC1D24*

(A) Profound deafness in three families cosegregates with the c.208G>T (p.Asp70Tyr) variant in *TBC1D24*. Representative chromatograms from wild-type and mutant sequences are shown. Altered nucleotides and affected residues are highlighted in gray. Genotypes of the participating family members are shown below each symbol in single-letter amino acid nomenclature. Individuals diagnosed with either seizures or epilepsy are highlighted by a gray rectangle. In family PKDF799, 10 of 11 deaf individuals have no history of seizures. Deaf female IV-13 had febrile seizures at the age of 8 years. In family DEM4587, there is no history of seizures in any of the eight clinically investigated family members, indicated by asterisks. Clinical re-evaluation of families DEM4221 and DEM4476 was not possible.

(B) Deaf individuals from family DEM4476 are homozygous for the c.878G>C (p.Arg293Pro) variant in *TBC1D24*, whereas normal-hearing parents and siblings of affected family members are heterozygous carriers of this mutation.

amino acid is not evolutionarily conserved and the substitution is predicted to not be detrimental by five in silico programs, including MutationTaster, PolyPhen-2, and SIFT. The synonymous variant c.720G>A (p.Ser240Ser) in *PRSS27* (MIM 608018) was not detected in 490 ethnically matched control chromosomes but has an allele frequency of 0.47% in the EVS (Table 1). A transition mutation, c.973C>T (p.Arg280Trp) in *THOC6* (MIM 615403), identified in family DEM4221 does not cosegregate with deafness, further refining the linkage interval of this family (Figure 1).

TBC1D24 was the only gene in which homozygous pathogenic mutations were found in the exome data sets of all three families (Table 1). Deaf members of families PKDF799 and DEM4221 are homozygous for a transversion mutation, c.208G>T, predicted to cause a p.Asp70Tyr substitution. Deaf members of family DEM4476 are homozygous for c.878G>C (p.Arg293Pro) in *TBC1D24*. We also Sanger sequenced the coding exons of *TBC1D24* in affected individuals from the fourth family, DEM4587, and found the c.208G>T (p.Asp70Tyr) mutation. The

c.208G>T mutation occurs on a haplotype spanning 477 kb shared among three families (Table S3). The two missense alleles of *TBC1D24* cosegregate with deafness in these families, are absent from the EVS and 1000 Genomes Project, and were not observed in at least 634 control chromosomes from ethnically matched individuals (Table 1). The substitutions p.Asp70Tyr and p.Arg293Pro affect residues conserved in orthologs of *TBC1D24* from *Drosophila melanogaster* to *Homo sapiens* (Figure 3A). The p.Asp70Tyr substitution is predicted to be “damaging” to *TBC1D24* function by MutationTaster, Mutation Assessor, LRT, SIFT, and PolyPhen-2. The p.Arg293Pro variant was assessed by SIFT as “tolerated” but was predicted to be “damaging” by the other four algorithms and is not reported in the EVS or 1000 Genomes Project. However, the EVS does contain two variants for the Arg293 codon: c.877C>T (p.Arg293Cys) and c.878G>A (p.Arg293His). In the ~6,500 EVS exomes, a heterozygous p.Arg293Cys substitution was detected once among 12,838 chromosomes, whereas heterozygosity for p.Arg293His was detected seven times among

Table 1. Homozygous Variants Identified within the *DFNB86* Interval

Gene	Variant ^a	mRNA Change ^b	Deduced Effect on Protein ^b	Family	Mutation Type	Allele Frequency in EVS ^c	Allele Frequency in Control Chromosomes
<i>CACNA1H</i>	g.1257427G>A	c.3060G>A (NM_001005407.1)	p.Ala1020Ala (NP_001005407.1)	PKDF799	synonymous	3/12,229	2/174
<i>IGFALS</i>	g.1841013C>T	c.1520G>A (NM_001146006.1)	p.Arg507His (NP_001139478.1)	DEM4476	missense	0/12,875	. ^d
<i>TBC1D24</i>	g.2546357G>T	c.208G>T (NM_001199107.1)	p.Asp70Tyr (NP_001186036.1)	PKDF799	missense	0/12,875	0/682
<i>TBC1D24</i>	g.2546357G>T	c.208G>T (NM_001199107.1)	p.Asp70Tyr (NP_001186036.1)	DEM4221	missense	0/12,875	0/682
<i>TBC1D24</i>	g.2547027G>C	c.878G>C (NM_001199107.1)	p.Arg293Pro (NP_001186036.1)	DEM4476	missense	0/12,875	0/634
<i>PRSS27</i>	g.2762774C>T	c.720G>A (NM_031948.3)	p.Ser240Ser (NP_114154.1)	PKDF799	synonymous	61/12,875	0/490
<i>PRSS27</i>	g.2762774C>T	c.720G>A (NM_031948.3)	p.Ser240Ser (NP_114154.1)	DEM4221	synonymous	61/12,875	0/490
<i>SRRM2</i>	g.2819161_2819163delTCT	c.7897_7899delTCT (NM_016333.3)	p.Ser2633del (NP_057417.3)	DEM4476	in-frame deletion ^e	49/12,396	1/290
<i>THOC6</i>	g.3077605C>T	c.973C>T (NM_024339.3)	p.Arg280Trp ^f (NP_077315.2)	DEM4221	missense	0/12,875	0/148

^aCoordinates are based on the hg19 human reference sequence (UCSC Genome Browser).

^bRefSeq accession numbers are shown in parentheses.

^cNHLBI Exome Sequencing Project (ESP) Exome Variant Server.

^dArg507 is not conserved in mouse, rat, or dog. The substitution of His for Arg507 is predicted to be neutral by MutationTaster, PolyPhen-2, SIFT, LRT, and MutationAssessor.

^eThis deletion of three nucleotides is one of several similar indels previously reported in the EVS as common SNPs resulting in deletion or insertion of one or more serine residues in a stretch of 42 consecutive serines.

^fDoes not cosegregate with deafness in family DEM4221.

12,828 chromosomes. Both of these rare variants are predicted by in silico programs to be damaging to *TBC1D24* function, suggesting a crucial role for Arg293 in the normal function of *TBC1D24*.

Human *TBC1D24* has eight exons, and the longest mRNA encodes a protein of 559 amino acids (Figure 3B). In mammals, *TBC1D24* is one of 42 TBC (Tre-2, Bub2, Cdc16)-domain-containing family members.²⁰ TBC domains have approximately 200 residues that are conserved in amino acid sequence from yeast to human.²¹ The p.Asp70Tyr substitution is located within the TBC domain of *TBC1D24* (Figure 3B). Although the crystal structure of *TBC1D24* is not known, the sequence can be compared with that of other TBC domains that have X-ray crystal structures available. Comparison with the sequence of *TBC1D4* suggests that the altered residue Asp70 is analogous to residue Asp950 of *TBC1D4* (3QYB).²² This residue is located in a loop between two helices, termed α 3 and α 4, in the *TBC1D4* structure. The boundaries of this loop vary significantly between different TBC domains, and high B-factors indicate a relatively large degree of flexibility or disorder in this region. Although it is possible that the loop might be the site of interaction with a different region of *TBC1D24* or a binding region for another protein, no specific roles for the loop have yet been identified.

Some TBC-domain-containing proteins have been shown to function as GTPase-activating proteins (GAPs),

which accelerate the intrinsic rate of GTP hydrolysis of specific Rab-GTPases.²³ When the GTP of a Rab-GTPase is hydrolyzed to GDP by a GAP, the protein becomes inactive until the GDP is exchanged for GTP by another class of regulatory proteins termed guanine nucleotide exchange factors.²⁰ Thus, GAPs are involved in the regulation of numerous membrane-trafficking and sorting processes of vesicles by modulating the activity of Rab-GTPases.²⁰ A direct Rab-GTPase target of *TBC1D24* has not been reported and might not exist. In yeast, the TBC-domain-containing protein Gyp1p (RefSeq accession number NP_014713.1) has been demonstrated to require an arginine at residue 343 for its Rab-GAP-stimulating activity.²³ Clustal Omega alignment of the TBC domains of yeast Gyp1p and human *TBC1D24* shows that there is a glutamine rather than a catalytic arginine at residue 100 of *TBC1D24*, suggesting that *TBC1D24* lacks Rab-GAP GTPase-stimulating activity.²⁰ However, ARF6 (ADP-ribosylation factor 6) was reported to be a partner of *TBC1D24* and could provide GTPase activity.²⁴

To begin to understand wild-type *TBC1D24* function in normal hearing, we examined its pattern of expression, alternative transcript splicing, and immunolocalization of *TBC1D24* in the mouse inner ear. Although not previously reported in the auditory system, human *TBC1D24* is expressed in a variety of tissues, including the heart, liver, kidney, stomach, lungs, and brain.²⁴ The NCBI

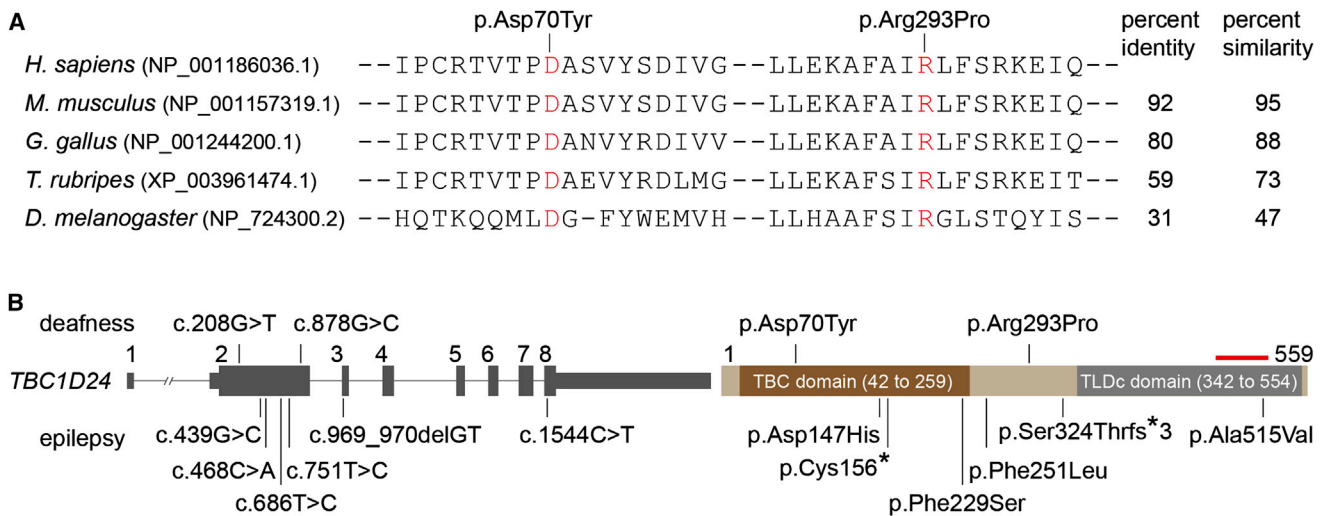


Figure 3. Structure of *TBC1D24* and Amino Acid Conservation of the Affected *TBC1D24* Residues

(A) ClustalW2 alignment of *TBC1D24* orthologs shows that the two DFNB86-associated mutations affect conserved residues (red font). Amino acid residue identity and similarity percentages were calculated for full-length *TBC1D24*.

(B) Graphic representation of *TBC1D24* structure (left) and its encoded protein (right). The red bar above the TLDC domain represents the epitope for the commercial antibody (Abcam, ab101933) that we used in Figure 4. DFNB86-associated mutations are shown above the diagram, and epilepsy-associated mutations are shown below. Tall and short gray boxes represent coding exons and UTRs of the transcribed gene, respectively. Horizontal lines joining the gray boxes denote introns. The longest isoform of *TBC1D24* was used as the reference sequence for mutation nomenclature (RefSeq NM_001199107.1 and NP_001186036.1). c. 1544C>T (p.Ala515Val) is renamed here according to the longest isoform but was originally reported as c.1526C>T (p.Ala509Val) on the basis of a shorter isoform of *TBC1D24* (Falace et al.²⁴).

Gene database lists eight splice variants composing two protein isoforms (a and b) of mouse *Tbc1d24* (NCBI Gene ID 224617). Isoforms a and b are predicted to encode 561 amino acids (63.2 kDa) and 555 amino acids (62.6 kDa), respectively. We investigated mouse *Tbc1d24* mRNA expression after preparing a cDNA library from P12 mouse inner-ear tissue. We used forward and reverse PCR primers complementary to the 5' UTR and 3' UTR of the full-length transcript to amplify and characterize splice isoforms of this gene (Figure S1). Agarose gel electrophoresis separated the PCR product into five bands of distinct sizes (data not shown) that were individually isolated, cloned, and Sanger sequenced. We detected nine alternatively spliced transcripts, including the previously reported isoforms a and b, of protein-coding exons of *Tbc1d24* (Figure S1). Isoforms a–e include large exon 5, which encodes 57% of the full-length protein, including the translation initiation codon. However, exon 5 is not included in isoforms f–i. If translated, these shorter transcripts would utilize in-frame ATG codons that do not satisfy the consensus (–3A and +4G) for a Kozak start site,²⁵ which is not an absolute requirement for translation initiation. If these short isoforms are also expressed in humans, they would not be affected by the two *TBC1D24* missense mutations associated with DFNB86 deafness.

Immunoblot analysis of P12 mouse brain and cochlea lysates with the use of a commercially available *TBC1D24* antibody (Abcam, ab101933) revealed a signal at 60 kDa, consistent with the expected size for the two previously reported *TBC1D24* isoforms. There was also a signal at

20 kDa in untransfected COS-7 cells and in mouse brain and cochlear lysates. The 20 kDa signal might be the short isoform f, which includes the epitope recognized by *TBC1D24* antibody ab101933 (Figure S1). To determine whether this antibody recognizes *TBC1D24*, we included a lysate from COS-7 cells transfected with an expression vector encoding pEGFP-*TBC1D24* (full length) in the immunoblot analysis, and the expected product corresponding to EGFP-*TBC1D24* (26.9 + 63.2 kDa) was detected at approximately 90 kDa (Figure S1).

To investigate *TBC1D24* localization in P30 mouse inner-ear, we immunolabeled²⁶ cryosections with the *TBC1D24* antibody and counterstained them with DAPI and rhodamine phalloidin. The strong signal for *TBC1D24* was observed in spiral ganglion cells, a collection of neurons critical for hearing and balance (Figure 4).

In addition to a TBC domain, the only other predicted domain in *TBC1D24* is a C-terminal TLDC domain (TBC, LysM, domain catalytic). Neither of the two *TBC1D24* missense mutations associated with deafness alters the TLDC domain. There are four other human TLDC-domain-containing proteins, including NCOA7 and OXR1, both of which have been demonstrated to defend cells against oxidative stress.^{27,28} Interestingly, the mouse OXR1 TLDC domain alone is sufficient to protect granule cells of the cerebellum against oxidative stress.²⁷ Clustal Omega alignment of the 163 residues of the TLDC domain of mouse OXR1 shows 66% similarity to the TLDC domain of human *TBC1D24*. Given the reported role of TLDC domains, an additional wild-type function of *TBC1D24*

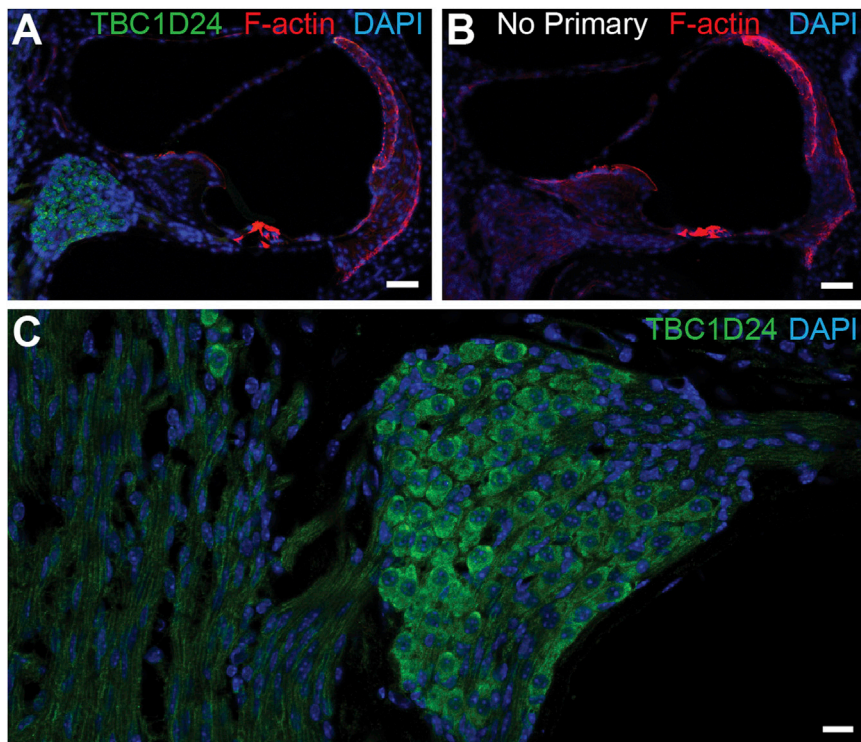


Figure 4. TBC1D24 Is Found in Spiral Ganglion Neurons of the Mouse Inner Ear (A) TBC1D24 antibody binds to spiral ganglion cells in the P30 cochlea. (B) The corresponding signal is absent in a control experiment with no added primary antibody. (C) A higher-magnification image reveals TBC1D24 antibody binding to the cell body and axonal projections of spiral ganglion. Scale bars in (A) and (B) represent 40 μm , whereas that in (C) represents 10 μm .

might be to help safeguard spiral ganglion neurons against oxidative stress.

Assuming that p.Asp70Tyr and p.Arg293Pro cause deafness as a result of an altered function of TBC1D24 in the spiral ganglion neurons of the inner ear, DFNB86 deafness might be an auditory neuropathy spectrum disorder.^{29–31} These disorders are audiological defined by abnormal auditory brainstem response (ABR) waveforms and interpeak intervals in combination with normal otoacoustic emissions (OAEs).³² These findings reflect an underlying lesion in the afferent auditory pathway, which includes the auditory nerve, the afferent synapse with the inner hair cell, or the inner hair cell itself. In the absence of afferent stimulation, the remaining hair cells and organ of Corti can eventually degenerate secondarily. Unfortunately, we were not able to obtain OAE and ABR data from any of our subjects. However, mouse models of DFNB86 might allow testing of the hypothesis that the primary lesion in TBC1D24 affects spiral ganglion neurons.

Four independent publications have described families in which epilepsy, with varying degrees of severity, segregates with recessive mutations in *TBC1D24* (Figure 3B and Table 2). Individuals from an Italian family affected by familial infantile myoclonic epilepsy (FIME [MIM 605021]) were found to be compound heterozygous for c.439G>C (p.Asp147His) and c.1544C>T (p.Ala515Val) in *TBC1D24*.²⁴ In another family (ethnicity not reported), two siblings presented with malignant migrating partial seizures of infancy (MIM 605021) as a result of compound heterozygosity for c.468C>A (p.Cys156*) and c.686T>C (p.Phe229Ser) in *TBC1D24*.³⁵ In an Arab family, four siblings with a focal epilepsy and intellectual disability syn-

drome (MIM 605021) were homozygous for a c.751T>C (p.Phe251Leu) allele of *TBC1D24*.³⁴ Finally, in a family from Turkey, a homozygous 2-nucleotide deletion located in cassette exon 3 of *TBC1D24* (Figure 3B) caused myoclonic epilepsy and severe neurodegeneration culminating in childhood death between the ages of 1.5 and 7 years.³³

Do mutations in *TBC1D24* cause either epilepsy without hearing loss or hearing loss without epilepsy? The four reports documenting seizure disorders do not mention hearing loss in epileptic individuals.^{24,33–35} In the four DFNB86-affected families, epilepsy might initially have gone undetected given that a family history of seizures was not an element of ascertainment. Therefore, we reassessed 15 deaf subjects in our study for a history of epilepsy. We were able to obtain clinical data for only two of the four families (DEM4587 and PKDF799, Figure 2). In family DEM4587, no history of seizures was reported for the eight individuals indicated by asterisks in Figure 2A. Brain MRI and electroencephalography (EEG) of deaf individuals IV-1 and IV-2 were normal. In family PKDF799, a history regarding seizures and epilepsy was obtained from 25 individuals, and EEG was performed for individuals III-12, IV-8, IV-12, IV-14, IV-15, and IV-17 (Figure 2A). EEG of a 16-year old individual with normal hearing (IV-12) was consistent with potential seizure activity, but he reported not ever having a seizure. Individual IV-8 (18 years old), who has normal hearing, has seizures that began when she was approximately 3 years of age and now occur two to three times a month. At the age 8 years, a third family member, an 18-year-old deaf individual (IV-13) had seizures that, according to her parents, were associated with a fever, which is common in children with hyperpyrexia. She has not had a seizure since then. The remaining 22 evaluated family members have no history of seizures. Individuals IV-8 and IV-12 are heterozygous for c.208G>T (p.Asp70Tyr) in *TBC1D24*, and their audiograms showed normal hearing thresholds (data not shown). For p.Asp70Tyr carrier IV-8, all eight exons of *TBC1D24* were sequenced and no other variants of this gene were found. To date, only recessive

Table 2. *TBC1D24* Mutations and Associated Phenotypes

Origin	Phenotype	Mutation ^a	Zygoty in Affected Individuals	Reference
Pakistan	nonsyndromic deafness	c.208G>T (p.Asp70Tyr)	homozygous	this study
Pakistan	nonsyndromic deafness	c.878G>C (p.Arg293Pro)	homozygous	this study
Turkey	myoclonic epilepsy, dystonia, developmental and neurological disability, childhood lethality	c.969_970delGT (p.Ser324Thrfs*3)	homozygous	Guven et al. ³³
Israel	focal epilepsy and intellectual disability	c.751T>C (p.Phe251Leu)	homozygous	Corbett et al. ³⁴
Italy	myoclonic and generalized tonic-clonic seizures, photosensitivity	c.439G>C (p.Asp147His) c.1544C>T (p.Ala515Val) ^b	compound heterozygous	Falace et al. ²⁴
Not reported	malignant migrating partial seizures of infancy	c.468C>A (p.Cys156*) c.686T>C (p.Phe229Ser)	compound heterozygous	Milh et al. ³⁵

^aNomenclature in this table is based on the longest isoform (RefSeq NM_001199107.1 and NP_001186036.1) of *TBC1D24*.

^bReported as p.Ala509Val on the basis of a shorter isoform (RefSeq NP_065756.1) of *TBC1D24*.

mutations in *TBC1D24* have been associated with epilepsy.^{24,33–35} Only one of two individuals with epilepsy in family PKDF799 is both deaf and homozygous for a mutation in *TBC1D24*. The World Health Organization reports that the world prevalence of epilepsy is 0.4%–1%. Given the high prevalence of epilepsy, especially in developing countries, we suspect that this association is a coincidence.

The localization of *TBC1D24* in neurons links two distinct phenotypes, epilepsy and deafness. In the inner ear, *TBC1D24* was immunolocalized predominantly in spiral ganglion neurons (Figure 4), whereas in the brain, *TBC1D24* mRNA was localized by in situ hybridization to the hippocampus and cortex.²⁴ Falace and coauthors also reported that ARF6, a member of a family with GTPase activity, is a partner of *TBC1D24* and is involved in neurite outgrowth.²⁴ ARF6 is also a component of a complex with scaffold protein GAB1 (GRB2-associated binding protein 1), GGA3 (golgi-associated, gamma adaptin ear-containing Arf-binding protein 3), and CRK (v-crk avian sarcoma virus CT10 oncogene homolog). This complex mediates the MET (receptor tyrosine kinase) internalization and recycling pathway^{36,37} only after MET is activated by its ligand HGF. We previously reported that non-protein-coding recessive mutant alleles of *HGF* cause human nonsyndromic deafness DFNB39.¹⁴ The p.Asp147His epilepsy-associated substitution, located in the TBC domain of *TBC1D24*, reduces its binding to ARF6.²⁴ It remains to be determined whether ARF6 is also localized to spiral ganglion neurons and whether or not the deafness-associated substitution p.Asp70Tyr alters ARF6 regulatory activity.

Assuming that there is normal hearing in epileptic individuals with recessive mutations in *TBC1D24*,^{24,33–35} it is intriguing that different recessive alleles of *TBC1D24* cause two distinct phenotypes, deafness and epilepsy. One possibility is that genetic background modifies the phenotypic outcome of *TBC1D24* mutations. Alternatively, differences in mutant alleles of *TBC1D24* might directly account for the different phenotypic outcomes, epilepsy and deafness.

Mouse *Tbc1d24* knockin alleles engineered to model the human mutations could address these questions.

Supplemental Data

Supplemental Data include one figure and three tables and can be found with this article online at <http://www.cell.com/AJHG/>.

Acknowledgments

We thank the subjects who participated in this study, as well as Dennis Drayna, Katie S. Kindt, and Julie Schultz for their critiques of our manuscript. We also thank Barbara Zwiesler, Elizabeth Wilson, Roger Murayi, and Hashim Raza for technical assistance. This study was supported by National Institutes of Health (NIH) National Institute on Deafness and Other Communication Disorders (NIDCD) grants R01 DC011651 and R01 DC003594 to S.M.L., the Higher Education Commission, the Ministry of Science and Technology of Pakistan (Sh.R. and W.A.), the International Center for Genetic Engineering and Biotechnology in Trieste (CRP/PAK08-01 contract 08/009 to Sh.R.), and the NIH National Human Genome Research Institute and National Heart, Lung, and Blood Institute (NHLBI) (U54 HG006493 to the University of Washington Center for Mendelian Genomics). Genotyping for S.M.L. was performed at the Center for Inherited Disease Research, which is funded by the NIH (contract number N01 HG65403 to Johns Hopkins University). N.B. was supported by NHLBI intramural funds HL004232 to James Sellers. Work at the NIH NIDCD was supported by intramural funds DC000060-12 to A.J.G. and DC000039-16 to T.B.F.

Received: October 31, 2013

Accepted: December 6, 2013

Published: January 2, 2014

Web Resources

The URLs for data presented herein are as follows:

1000 Genomes, <http://www.1000genomes.org>
 ANNOVAR, <http://www.openbioinformatics.org/annovar/>
 Clustal Omega, <http://www.ebi.ac.uk/Tools/msa/clustalo/>

Hereditary Hearing Loss Homepage, <http://hereditaryhearingloss.org/>
 MutationAssessor, <http://mutationassessor.org/>
 MutationTaster, <http://www.mutationtaster.org/>
 NCBI Gene, <http://www.ncbi.nlm.nih.gov/gene>
 NHLBI Exome Sequencing Project (ESP) Exome Variant Server, <http://evs.gs.washington.edu/EVS/>
 Online Mendelian Inheritance in Man (OMIM), <http://www.omim.org>
 PolyPhen-2, <http://genetics.bwh.harvard.edu/pph2/>
 Primer3, <http://bioinfo.ut.ee/primer3-0.4.0/primer3/>
 Primer-BLAST, <http://www.ncbi.nlm.nih.gov/tools/primer-blast/>
 SeattleSeq Annotation 137, <http://snp.gs.washington.edu/SeattleSeqAnnotation137/>
 SIFT, <http://sift.bii.a-star.edu.sg>
 UCSC Genome Browser, <http://genome.ucsc.edu>

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