The American Journal of Human Genetics, Volume *94*

Supplemental Data

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

Atteeq U. Rehman, Regie Lyn P. Santos-Cortez, Robert J. Morell, Meghan C. Drummond, Taku Ito, Kwanghyuk Lee, Asma A. Khan, Muhammad Asim R. Basra, Naveed Wasif, Muhammad Ayub, Rana A. Ali, Syed I. Raza, University of Washington Center for Mendelian Genomics, Deborah A. Nickerson, Jay Shendure, Michael Bamshad, Saima Riazuddin, Neil Billington, Shaheen N. Khan, Penelope L. Friedman, Andrew J. Griffith, Wasim Ahmad, Sheikh Riazuddin, Suzanne M. Leal, and Thomas B. Friedman

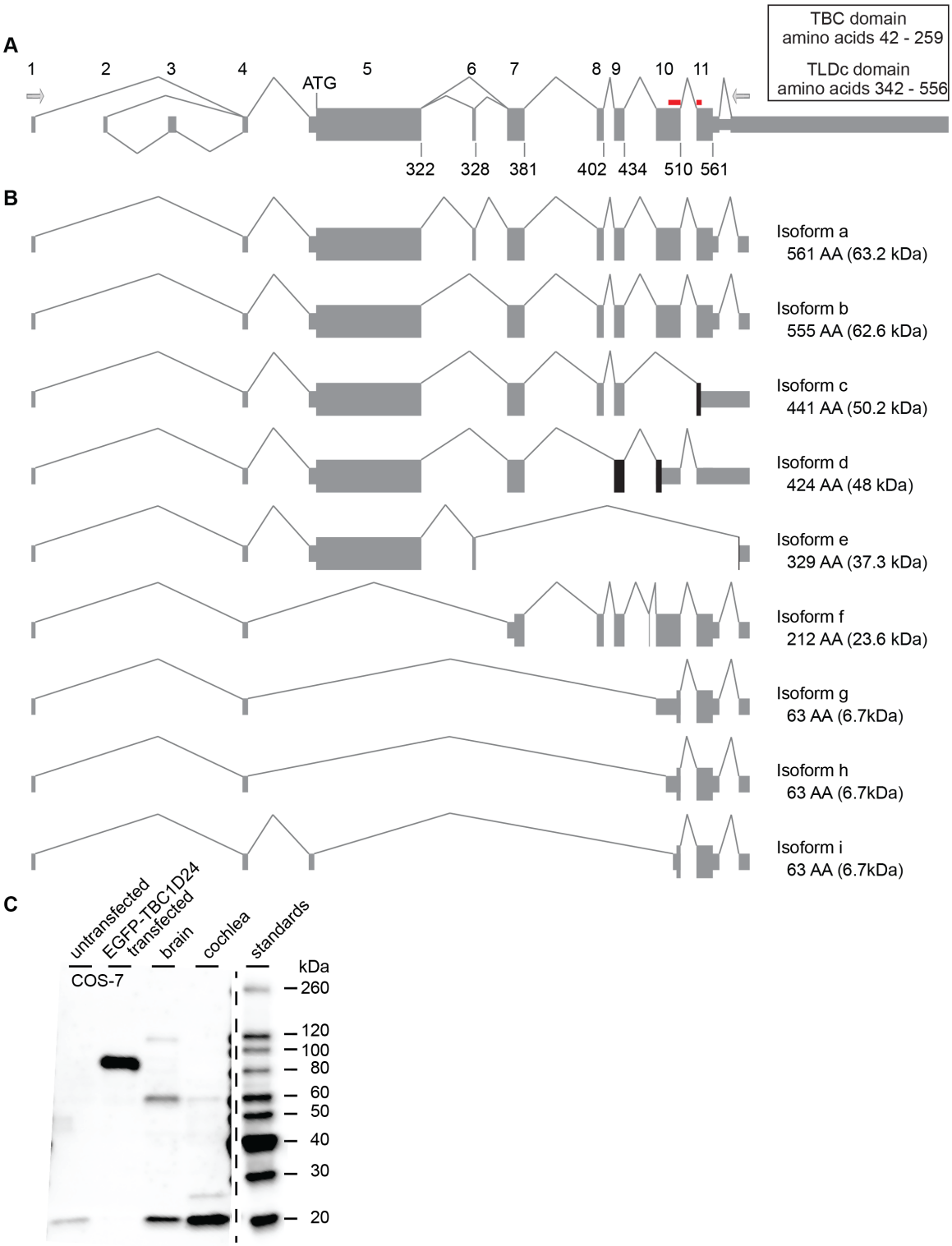
A
TBC domain
amino acids 42 - 259
TLDc domain
amino acids 342 - 556
1
2
3
4
ATG
5
6
7
8
9
10
11
322
328
381
402
434
510
561
B
Isoform a
561 AA (63.2 kDa)
Isoform b
555 AA (62.6 kDa)
Isoform c
441 AA (50.2 kDa)
Isoform d
424 AA (48 kDa)
Isoform e
329 AA (37.3 kDa)
Isoform f
212 AA (23.6 kDa)
Isoform g
63 AA (6.7kDa)
Isoform h
63 AA (6.7kDa)
Isoform i
63 AA (6.7kDa)
C
untransfected
COS-7
EGFP-TBC1D24
transfected
brain
cochlea
standards
kDa
260
120
100
80
60
50
40
30
20

Figure S1. *Tbc1d24* Exon Splicing Patterns Identified in the Mouse Inner Ear

(A) Spliced transcripts of mouse *Tbc1d24* annotated in the NCBI Gene database on October 30, 2013. Thick bars represent exons. Thin bars are UTRs. Angled lines joining the exon boundaries depict mRNA splicing events. The residue number encoded by the last codon of each exon is shown below the diagram. The two red bars represent the location of exonic sequences that encode the epitope for a commercial antibody (Abcam, ab101933), which was used for western blot analyses and immunolocalization experiments (Figure 4). The two arrows indicate the forward and reverse PCR primers used to amplify *Tbc1d24* transcripts from a mouse inner ear cDNA library.

(B) Alternatively spliced transcripts of *Tbc1d24* detected in cDNA prepared from a postnatal day 12 mouse inner ear. The PCR primer pair (arrows, panel A) used in this assay will not amplify transcripts that contain either exons 2 or 3 or both as well as the portion of the 3' UTR downstream of the primer in exon 11. In addition to the previously known isoforms a and b, we identified seven additional putative protein isoforms arising from splice variants expressed in the inner ear of mouse. Black bars indicate exons that encode deduced residues in a reading frame different from the other isoforms of TBC1D24 described here.

(C) Western blot analysis of postnatal day 12 (P12) mouse brain and cochlea lysates reveal anti-TBC1D24 antibody binding at 60 kDa and 20 kDa. A rabbit polyclonal anti-TBC1D24 (Abcam) was used at a dilution of 1:1,000 in ELC Plus blocking solution (GE) and a HRP-conjugated goat anti-rabbit-IgG (Sigma) was used at 1:100,000 dilution in blocking solution. 0.5 micrograms of lysate from transfected and untransfected COS-7 cells and 15 micrograms of total tissue lysate from mouse brain and mouse cochlea were

size separated on 4-20% SDS PAGE. The antibody detects a product of the predicted size (90 kDa) in the lysate of COS-7 cells expressing EGFP-TBC1D24, but not in an untransfected COS-7 cell lysate. All lysates were prepared in RIPA supplemented with HALT Protease Inhibitor Cocktail (Pierce).

Table S1. Inbreeding Coefficients Based on Pedigree Structure

Family	Affected Individual[a]	Parental Relations	[b]Inbreeding Coefficient
PKDF799	III-11	Unrelated	0
	III-13	Unrelated	0
	IV-13	First cousins	0.0625
	IV-14	First cousins	0.0625
	IV-15	First cousins	0.0625
	IV-16	First cousins	0.0625
	IV-17	First cousins	0.0625
	IV-22	First cousins	0.0625
	IV-23	First cousins	0.0625
	IV-30	Double first-cousins	0.25
	IV-31	Double first-cousins	0.25
	Median		0.0625
DEM4221	II-7	Unrelated	0
	III-2	Unrelated	0
	III-6	Unrelated	0
	IV-3	Unrelated	0

	IV-5	Unrelated	0
	IV-10	Second cousins	0.015625
	IV-11	Second cousins	0.015625
	V-1	First cousins	0.0625
	V-2	Second cousins	0.015625
	Median		0
DEM4587	IV-1	First cousins	0.0625
	IV-2	First cousins	0.0625
	IV-3	First cousins	0.0625
	IV-5	First cousins	0.0625
	Median		0.0625
DEM4476	IV-2	First cousins	0.0625
	IV-4	First cousins	0.0625
	IV-5	First cousins	0.0625
	IV-6	First cousins	0.0625
	IV-7	First cousins	0.0625
	IV-8	First cousins	0.0625
	IV-9	First cousins	0.0625
	Median		0.0625

[a]Generation and ID for each affected individual is based on Figure 2.

[b]Cryptic consanguinity for each of these families is highly plausible, and we do not have enough genetic information to derive inbreeding coefficients. Therefore, the inbreeding coefficient for each family is based on pedigree structure.

Table S2. Summary of Exome Sequencing Data From Affected Members of Three Families Segregating Deafness Linked to *DFNB86*

Category	PKDF799 IV-23	DEM4221 IV-11	DEM4476 IV-6
Targeted exome	45 Mb	64 Mb	64 Mb
Average depth of coverage	51.27	56.98	50.28
Percentage coverage >10X	91.74	94.94	94.36
Total variants	22,186	25,455	25,490
Putative pathogenic variants[a]	521	642	610
Refined *DFNB86* linkage interval[b]			
Average depth of coverage	41.52	64.78	39.00
Percentage coverage >10x	79.29	92.90	84.84
Total variants	45	50	61
Putative pathogenic variants[a]	3	3	3

[a]Homozygous missense, synonymous, truncating, or splice site variants which are not in a duplicated region and have a minor allele frequency < 0.01 in 1000 Genomes and NHLBI-ESP6500.

[b]2.05-Mb interval between genetic markers *rs2072042* and *D16S3070*.

Table S3. SNP Genotypes Across a 477 Kb Genomic Interval Encompassing *TBC1D24*

SNP Marker	Position (hg19)	[a]PKDF799	DEM4221	DEM4587	DEM4476
rs26840	2,285,357	T/T	T/T	T/T	C/C
rs374015103	2,499,786	TCCCCTCCA/ TCCCCTCCA	TCCCCTCCA/ TCCCCTCCA	TCCCCTCCA/ TCCCCTCCA	[b]-/-
[a]c.208G>T	2,546,357	T/T	T/T	T/T	G/G
rs76267944	2,551,015	T/T	T/T	T/T	C/C
rs144374231	2,762,774	T/T	T/T	T/T	C/C
unknown	2,762,878	T/T	T/T	T/T	C/C

[a]Families PKDF799, DEM4221, and DEM4587 co-segregate deafness with the c.208G>T allele (p.Asp70Tyr) of *TBC1D24* whereas family DEM4476 co-segregates deafness with c.878G>C (p.Arg293Pro) of *TBC1D24*.

[b]The allele of this SNP without the 9 bp insertion.