EDITOR'S CHOICE

Nonsense mutation of the stereociliar membrane protein gene *PTPRQ* in human hearing loss *DFNB84*

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ABSTRACT

Background Moderate to severe prelingual hearing impairment (*DFNB84*) was observed in an extended consanguineous Palestinian kindred. All affected relatives shared a 12.5 MB homozygous haplotype on chromosome 12q21 with lod score 4.30. This homozygous region harbours the protein tyrosine phosphatase receptor Q gene *PTPRQ*, which is known to be essential to hearing in mouse.

Methods Candidate genes in the 12.5 MB homozygous region were characterized genomically and sequenced in deaf and hearing relatives in the family.

Results Sequence of *PTPRQ* in affected individuals in the extended kindred revealed c.1285C→T, leading to p.Gln429Stop. This nonsense mutation co-segregated with hearing loss in the family and was homozygous in all affected relatives. The mutation did not appear among 288 Palestinian controls (576 chromosomes), all adults with normal hearing. No homozygous mutations in *PTPRQ* appeared in any of 218 other probands with hearing loss.

Conclusion Identification of the *DFNB84* gene represents the first identification of *PTPRQ* mutation in human hearing loss.

Hair cells of the inner ear are specialised mechanoreceptor cells that detect sound and head movement. The mechanical to electrical transduction is accomplished specifically by the hair bundle, comprised of approximately 100 actin filled stereocilia. Proteins of the stereociliar membrane include ion channels, chemoreceptors and cell adhesion molecules. One stereociliar membrane protein is PTPRQ (protein tyrosine phosphatase receptor type Q), which includes an extracellular domain containing 18 fibronectin III (FNIII) repeats, a membrane spanning domain, and a cytoplasmic domain with phosphatidylinositol phosphatase activity.[1 2] Mutation of *Ptprq* in the mouse causes deafness associated with disrupted stereociliar bundles.[3] Here we report the first human mutation of *PTPRQ* in an extended kindred with inherited hearing loss.

METHODS

Clinical evaluation

Children with prelingual, bilateral hearing loss were ascertained through Eftah School for the Deaf in Bethlehem. Informed consent was obtained from parents and assent from older children. The project was approved by the Human Subjects Committee of Bethlehem University and by the Human Subjects Division of the University of Washington. Audiological exams were performed on all affected family members at the Dar Al-Kalima Health and Wellness Center in Bethlehem to exclude hearing loss due to infection or trauma, to evaluate severity and laterality of the hearing loss. Vision exams were carried out in the same visits to assess the possibility of Usher syndrome. Population controls comprised 288 Palestinian adults with normal hearing and came from two sources: (1) persons attending West Bank clinics for preventive health services (n=201); and (2) Palestinian individuals living in Israel who contributed DNA to the National Laboratory for the Genetics of Israeli Populations at Tel Aviv University (n=87).

Localisation of *DFNB84*

Homozygosity mapping with Affymetrix 250K single nucleotide polymorphism (SNP) arrays was performed as previously described.[4] Affected individuals in this study were children in two families, CN and DP. The longest deafness associated homozygous segments for these families were chr12: 74 498 486-93 289 627 in family CN and chr12: 65 434 594-86 977 739 in family DP. The SNP haplotypes in the shared region (chr12: 74 498 486-86 977 739) were identical between families CN and DP. This locus was designated *DFNB84* by the HUGO Gene Nomenclature Committee.[4]

Mutation analysis

The human RefSeq entry for *PTPRQ* (NM_001145026) was aligned to hg18 of the human genome. The genomic interval from *PTPTQ* intron 3 to intron 6 is inverted on hg18. This may represent an assembly error in the reference genome rather than a true structural variant.[5] Primers spanning each exon and approximately 150 bp of flanking intronic sequence were designed (additional table 1) and used to PCR amplify DNA from individuals with hearing loss and their hearing parents. PCR products were Sanger sequenced on an Applied Biosystems 3730xl as previously described.[6]

RESULTS

The recessive deafness locus *DFNB84* was identified by SNP based homozygosity mapping in families CN and DP.[5] Because hearing loss of affected members of families CN and DP mapped to the same homozygous region of chromosome 12, family members shared historical information to determine how they might be related. They discovered that all participants from families CN and DP are members of the same extended kindred, henceforth referred to as family CN/DP (figure 1A). All affected individuals in family CN/DP shared

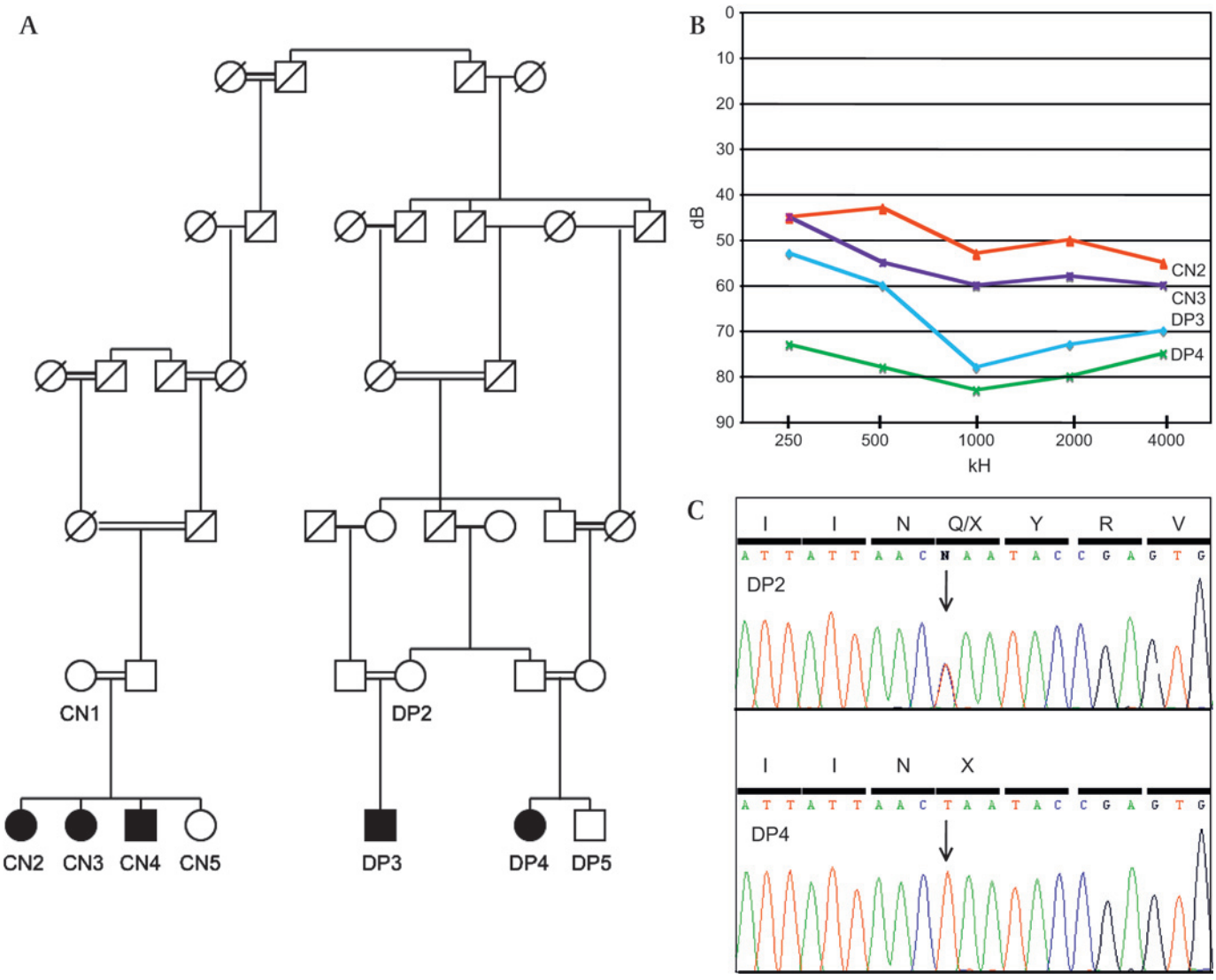

Figure 1 Inherited hearing loss in family CN/DP. (A) Pedigree of the extended kindred CN/DP, in which autosomal recessive hearing loss is linked to the *DFNB84* locus. Hearing loss in affected individuals is indicated by filled symbols. Double bars between parents indicate consanguineous marriages. (B) Audiometric profiles for hearing impaired individuals from family CN/DP. Audiograms for affected individuals in family CN/DP illustrating hearing thresholds for CN2 (age 11 years), CN3 (age 15 years), DP3 (age 5 years), and DP4 (age 14 years). (C) Nonsense mutation in *PTPRQ*. Sanger sequence trace of *PTPRQ* exon 9. Hearing individual DP2 is heterozygous for c.1285C→T (chr12:79 404 441) indicated by the arrow. This alteration is predicted to introduce stop codon TAA at codon 429, Q429X. Hearing impaired individual DP4 is homozygous for the c.1285 (p.Q429X) mutation.

a homozygous haplotype at chr12: 74 498 486-86 977 739 (NCBI Build hg18) with lod score 4.30 under a fully penetrant recessive model. The hearing loss phenotype in all hearing impaired individuals was moderate to severe with prelingual onset, with considerable variation among family members (figure 1B). There were no signs of conductive hearing loss, as measured by air and bone conduction thresholds. None of the affected individuals had any vision problems.

A promising candidate gene in the DFNB84 linkage interval was PTPRQ (protein tyrosine phosphatase receptor Q) located at bp 79 362 257-79 598 099 (hg18). PCR amplification and Sanger sequencing of the annotated 45 exons of PTPRQ revealed c.1285C→T (at chr12:79 404 441), which is predicted to introduce a premature truncation at codon 429, p.Q429X, in exon 9 (figure 1C). All hearing impaired individuals in the family were homozygous for this nonsense allele. A previously described polymorphism (rs61729287, C→G) is located at the same nucleotide as the family CN/DP nonsense mutation. However, the C→G variant corresponds to a conservative p.Q429E alteration.

Screening the nonsense allele in 288 Palestinian adults with normal hearing and 218 Palestinian probands with prelingual hearing loss did not reveal any other heterozygous or homozygous individuals, suggesting that c.1285C→T (p.Q429X) is a rare allele in the Palestinian West Bank population. Frequency of rs61729287 allele G was 0.03 among 218 unrelated Palestinian deaf probands and among 288 Palestinian controls, suggesting it is a benign polymorphism.

To search for additional deleterious *PTPRQ* alleles in the Palestinian deaf population we genotyped 218 individuals with prelingual hearing loss from consanguineous kindreds with microsatellites spanning 487 kb within and flanking *PTPRQ*. Homozygous genotypes at all four markers were observed in six probands. None of these probands shared the CN/DP haplotype. Full sequencing of *PTPRQ* in these six individuals did not reveal any additional mutations.

In characterising the *DFNB84* genomic region, we discovered 175 kb upstream of *PTPRQ* a previously unknown gene. Upon annotation in mouse cochlea, the gene proved homologous to Otogelin, mutations in which are responsible for the mouse *twister* phenotype.[7] We characterised this Otogelin-like gene (Otogl) and deposited it in GenBank (described in additional materials). Sequence of the human homologue *OTOGL* (additional figure 1) was wild type in all affected individuals of family CN/DP, excluding epistatic effects of this gene on the phenotype.

DISCUSSION

To date, 31 genes responsible for development and maintenance of hair cell bundles of the inner ear have been implicated in deafness in humans and mice.[8] In mouse, homozygous loss of function of *Ptprq* leads to deafness associated with absence of hair cells in the basal region of the cochlea.[3] $Ptprq^{-/-}$ mice age 3 months lack hair cells in the basal region of the cochlea, affecting high frequency hearing, but have no gross abnormalities in the apical end of the cochlea, affecting low frequency hearing.

The role of PTPRQ in the ear has been assessed by evaluating $Ptprq^{-/-}$ mice in the context of myosin VI function.[9] Myosin VI, which functions as either an actin based anchor or as a transporter, has been proposed to regulate localisation of PTPRQ in stereociliar membranes.[1] Myosin VI and PTPRQ co-localise in the stereocilia, suggesting that the two proteins interact. In the absence of myosin VI, PTPRQ is distributed along the entire length of the stereocilia. In *Ptprq*-null mice, stereocilia in the apical and middle turn are fused, similar to the phenotype of Snell's waltzer myosin VI-null mice.[10] Taken together, these studies suggest that PTPRQ may have multiple roles: stabilising the membrane at the base of the stereocilia, regulating actin dynamics in stereocilia, and together with myosin VI, tethering the stereociliar membrane to the cytoskeleton. Given the conservation of these functions between humans and mice, it was to be expected that loss of function of *PTPRQ* in humans would lead to hearing loss. The present identification of mutant *PTPRQ* for the first time in hearing impaired humans demonstrates its clinical importance.

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Competing interests None

Patient consent Obtained.

Ethics approval This study was conducted with the approval of the Human Subjects Committee of Bethlehem University and by the Human Subjects Division of the University of Washington.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

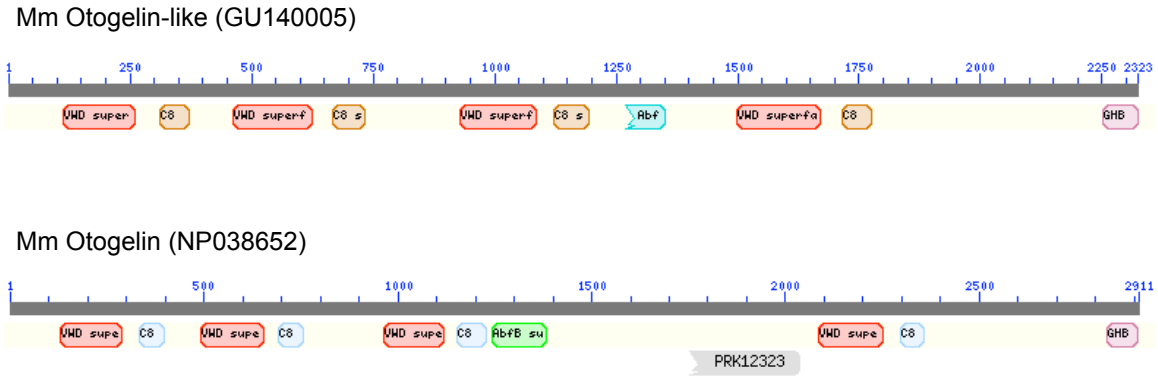
1. **Wright MB,** Hugo C, Seifert R, Disteche CM, Bowen-Pope DF. Proliferating and migrating mesangial cells responding to injury express a novel receptor protein-tyrosine phosphatase in experimental mesangial proliferative glomerulonephritis. *J Biol Chem* 1998;**273**:23929—37.
2. **Oganesian A,** Poot M, Daum G, Coats SA, Wright MB, Seifert RA, Bowen-Pope DF. Protein tyrosine phosphatase RQ is a phosphatidylinositol phosphatase that can regulate cell survival and proliferation. *Proc Natl Acad Sci U S A* 2003;**100**:7563—8.
3. **Goodyear RJ,** Legan PK, Wright MB, Marcotti W, Oganesian A, Coats SA, Booth CJ, Kros CJ, Seifert RA, Bowen-Pope DF, Richardson GP. A receptor-like inositol lipid phosphatase is required for the maturation of developing cochlear hair bundles. *J Neurosci* 2003;**23**:9208—19.
4. **Shahin H,** Walsh T, Abu Rayyan A, Lee MK, Higgins J, Dickel D, Lewis K, Thompson J, Carl Baker C, Nord AS, Stray S, Gurwitz D, Avraham KB, King M-C, Kanaan M. Five novel loci for inherited hearing loss mapped by SNP-based homozygosity profiles in Palestinian families. *Eur J Hum Genet*. Published Online First: 4 November 2009. PMID 19888295. doi:10.1038/ejhg.2009.190
5. **Kidd JM,** Cooper GM, Donahue WF, Hayden HS, Sampas N, Graves T, Hansen N, Teague B, Alkan C, Antonacci F, Haugen E, Zerr T, Yamada NA, Tsang P, Newman TL, Tüzün E, Cheng Z, Ebling HM, Tusneem N, David R, Gillett W, Phelps KA, Weaver M, Saranga D, Brand A, Tao W, Gustafson E, McKernan K, Chen L, Malig M, Smith JD, Korn JM, McCarroll SA, Altshuler DA, Peiffer DA, Dorschner M, Stamatoyannopoulos J, Schwartz D, Nickerson DA, Mullikin JC, Wilson RK, Bruhn L, Olson MV, Kaul R, Smith DR, Eichler EE. Mapping and sequencing of structural variation from eight human genomes. *Nature* 2008;**453**:56—64.
6. **Shahin H,** Walsh T, Sobe T, Abu Saed J, Abu Rayan A, Lynch ED, Lee MK, Avraham KB, King MC, Kanaan M. Mutations in a novel isoform of TRIOBP that encodes a filamentous-actin binding protein are responsible for *DFNB28* recessive nonsyndromic hearing loss. *Am J Hum Genet* 2006;**78**:144—52.
7. **Simmler MC,** Zwaenepoel I, Verpy E, Guillaud L, Elbaz C, Petit C, Panthier JJ. Twister mutant mice are defective for otogelin, a component specific to inner ear acellular membranes. *Mamm Genome* 2000;**11**:960—6.
8. **Petit C,** Richardson GP. Linking genes underlying deafness to hair-bundle development and function. *Nat Neurosci* 2009;**6**:703—10.
9. **Sakaguchi H,** Tokita J, Naoz M, Bowen-Pope D, Gov NS, Kachar B. Dynamic compartmentalization of protein tyrosine phosphatase receptor Q at the proximal end of stereocilia: implication of myosin VI-based transport. *Cell Motil Cytoskeleton* 2008;**65**:528—38.
10. **Self T,** Sobe T, Copeland NG, Jenkins NA, Avraham KB, Steel KP. Role of myosin VI in the differentiation of cochlear hair cells. *Dev Biol* 1999;**214**:331—41.

Supplementary Table 1. Primers used to screen PTPRQ

	cDNA bp		hg18						flanking bp	
exon	5'	3'	start	end	forward	reverse	forward	reverse	5'	3'
1	1	54	79,362,257	79,362,310	GTTTAACAAAGAAAAGGCAG	AACTTACAAAACGTACTGCG	79,362,108	79,362,456	149	146
2	55	163	79,362,652	79,362,760	TAAAGTCATTTGGTAAAAAG	CCTTATTCTACACTGTTTTC	79,362,464	79,362,882	188	122
3	164	390	79,363,402	79,363,630	CCACATAGTATTTTTTTAATAGTAACAGAG	AATCTCAGGTGGGTGTGAGG	79,363,240	79,363,779	162	149
4			79,373,406	79,373,655	TTAGGGGAAAAAAGTTACTG	GCATAAAAGAAAAAGAAATG	79,373,293	79,373,750	113	95
5			79,374,825	79,375,024	AGTAATCAAATAATAAAACG	TAAAAATCTTACACAAATAG	79,374,667	79,375,153	158	129
6			79,376,664	79,376,733	ATTTTAAGGAATAAATTAGC	ATATCTTCATATTTGCCTTC	79,376,498	79,376,883	166	150
7	909	1039	79,386,618	79,386,748	TATTTTATTTTCCTTCTTTG	TCCTTATGACTTTCCTATTG	79,386,515	79,386,888	103	140
8	1040	1186	79,390,015	79,390,161	TTAACACTTGAATTGTTGTC	GTTCAGAATGAATTAGGTAG	79,389,917	79,390,307	98	146
9	1187	1359	79,402,343	79,402,515	CCTAGTCTGTTTTCTAATAG	ATAACCTAAAATGAAATATG	79,402,215	79,402,690	128	175
10	1360	1540	79,411,185	79,411,365	AATTTTGGAATCTAAAGAAG	TTAATAATATGGCTGAACTC	79,411,031	79,411,462	154	97
11	1541	1702	79,412,843	79,413,004	CTAACTTTAATAGAGGTGTG	CCAATATAACAAAATAGATG	79,412,702	79,413,166	141	162
12	1703	1882	79,413,102	79,413,281	ATTAGTGTATCTGCTTTCAC	ACAACAAAATATGAAGTGAC	79,412,932	79,413,409	170	128
13	1883	1990	79,413,909	79,414,016	TTTTAGGAGAGAACTTGAAG	ATTTTTATTGATGTTTGTTG	79,413,713	79,414,126	196	110
14	1991	2272	79,414,160	79,414,441	CATCTTTGTGAGAACTTCAG	ATGAGGGATTTTAGGTTATG	79,413,991	79,414,546	169	105
15	2273	2455	79,423,934	79,424,116	TTACATTCTAGTGAAGGTTC	TAGTATTCTTTCATGTGACC	79,423,818	79,424,284	116	168
16	2456	2557	79,424,479	79,424,580	TACTATTGCCAAAGAATGAC	CACTGAATGTAAATCTGATG	79,424,340	79,424,765	139	185
17	2558	2678	79,428,233	79,428,353	GCAAAATCTTCCTTTATC	TTCCTAATGTAGCACCAC	79,428,054	79,428,455	179	102
18	2679	2839	79,451,925	79,452,085	GCAAAATAAGTTAATTTACTAAAG	CATCACACAGCTAAAAAGTG	79,451,731	79,452,245	194	160
19	2840	2985	79,452,802	79,452,947	TTATGGATTTCATTTCTGAC	CTACACCGCATTTTTCTATG	79,452,650	79,453,104	152	157
20	2986	3154	79,457,686	79,457,854	ATTTCCAGTTTAATTGTTGC	GCAGTTTTCTTGAACAGAAG	79,457,518	79,458,044	168	190
21	3155	3445	79,459,465	79,459,755	GCTGATGATGTAATAGTTGC	ATGGGATTTGATTTTCTTAG	79,459,358	79,459,927	107	172
22	3446	3721	79,459,999	79,460,274	AATAATCTGCTAAGAAAATC	GCTGTTACAAATAGACAATC	79,459,899	79,460,387	100	113
23	3722	3873	79,460,640	79,460,791	TACTTTGGAACTACTCTGTC	AATTGTAGGAAATAAAGAAG	79,460,460	79,460,926	180	135
24	3874	4015	79,464,466	79,464,607	AAACTGAGCACACTCTATGG	TGATTTCACACAAGACCAAG	79,464,287	79,464,767	179	160
25	4016	4285	79,467,375	79,467,644	TGATTCTGTCTATGTTAATG	GCTTTGTATGTTTAGATAAG	79,467,210	79,467,749	165	105
26	4286	4609	79,506,039	79,506,362	TCTTCTCATTAAGTGTGGGG	TGAATTTCTCATTGGGTTTC	79,505,887	79,506,542	152	180
27	4610	4731	79,522,969	79,523,090	AATGCCTTTAGCACTCTGTG	GATTCAGTCCTCATACCCTG	79,522,820	79,523,276	149	186
28	4732	4918	79,528,349	79,528,535	CACTTTAGATTTCCCAGAGC	TCTTCCTTTTTGTCAGTTGC	79,528,198	79,528,694	151	159
29	4919	5163	79,531,502	79,531,746	GATTCTATAATGCGTCAGTC	ATCTCACAAAAAGATATGGG	79,531,342	79,531,862	160	116
30	5164	5230	79,534,110	79,534,176	TAATAGACTATATGCAGTTG	TATTTAACATGGTAATTCAC	79,533,925	79,534,374	185	198
31	5231	5389	79,537,294	79,537,452	ATTGCTAAATGTCTGCTG	TTACAAAGAAATGATTGG	79,537,132	79,537,615	162	163
32	5390	5612	79,538,064	79,538,286	TGTAACAGAGAAGAACAGGG	CTTATTGGAAAGATGAAAGG	79,537,890	79,538,380	174	94
33	5613	5686	79,539,971	79,540,044	ATTTCTCCTGATGATGATTC	ACAAACCTTCACAGGTACTC	79,539,817	79,540,238	154	194
34	5687	5786	79,550,102	79,550,201	TAGAAAAAGCAAGAAGACCC	ATTCAAGTAACTGTTGTGGC	79,549,903	79,550,297	199	96
35	5787	5915	79,552,855	79,552,983	AAAACAAGAAAAAGAAGTCG	AAGTGTGTAGAAGACGGAGG	79,552,687	79,553,136	168	153
36	5916	5942	79,566,807	79,566,833	AAAGGCATAGCACATTTTAC	CACCCCAGAGAGAGAGAAAG	79,566,687	79,567,020	120	187
37	5943	6024	79,567,498	79,567,579	TGAATACAAAGAAAGTGAGC	CTATCAAAACAATCAACCTG	79,567,301	79,567,724	197	145
38	6025	6115	79,570,654	79,570,744	TGTTTCCACAGTTATGATAG	AGTCACTGCCAATAAAATAC	79,570,518	79,570,877	136	133
39	6116	6192	79,575,895	79,575,971	AATAGTCATTGTCTCTGG	TTAGTATCTCTGTTAGGC	79,575,718	79,576,161	177	190
40	6193	6237	79,586,917	79,587,051	AGATTATCTAGTATTTACGG	TATACCACAGTTATTACGAC	79,586,782	79,587,193	135	142
41	6238	6453	79,587,249	79,587,374	ATTTCATCTAATACTGTGAG	GACATTGATACTAAAGTTGG	79,587,114	79,587,551	135	177
42	6454	6602	79,588,254	79,588,402	TGTAACAATAAAAATCTCTC	CAGAAAACATAAATCATACC	79,588,089	79,588,568	165	166
43	6603	6738	79,591,079	79,591,214	ACCATAAAAAATCCAAAGAC	TAATGTAGGAAAAGAAGGTG	79,590,923	79,591,335	156	121
44	6739	6862	79,596,512	79,596,635	TTTAGAAAGCCTCCTATTTG	CAACTTGGATTAGAGAAAAG	79,596,332	79,596,789	180	154
45	6863	8066	79,596,896	79,596,933	TTTTCTCTAATCCAAGTTGG	TATTCCAAAATATGGAGGAC	79,596,771	79,597,207	125	274

Characterization of the Otogelin-like transcript

The genomic region of mouse chromosome 10 orthologous to the human *DFNB84* genomic region includes inner ear EST BY752804. We performed 3' and 5' RACE of BY752804 with total RNA prepared from cochlea dissected from age P5 mice. RACE products were sub-cloned and sequenced and compared to the mouse genome mm9 alignment. RACE experiments generated a 6780bp transcript corresponding to an open reading frame of 2323 amino acids. Sequence and protein alignment of this transcript revealed homology to otogelin, 41% identical and 60% similar at the amino acid level (Supplementary Figure 1). We named the newly characterized gene Otogelin-like (*Otogl*) and deposited the complete transcript sequence in GenBank (accession number GU140005). The most 3' exon of *Otogl* is 45kb upstream of *Ptprq* exon 1. Mutations in the original otogelin are responsible for the mouse *twister* allele, which is characterized by deafness and severe balance defects.[1] otolegin-like may prove a useful candidate gene for other deafness loci that map to this genomic region.



Supplementary Figure 1. Alignment of conserved domains of Mm Otogelin and Mm Otogelin-like proteins. Similarity searches of the NCBI Entrez Protein Database where performed using CDART[2].

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[1] Simmler MC, Zwaenepoel I, Verpy E, Guillaud L, Elbaz C, Petit C, Panthier JJ. Twister mutant mice are defective for otogelin, a component specific to inner ear acellular membranes. Mamm. Genome 2000, 11: 960-966.

[2] Geer LY, Domrachev M, Lipman DJ, Bryant SH. CDART: protein homology by domain architecture. Genome Research 2002, 12: 1619-1623