



# 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.<sup>1 2</sup> Mutation of *Ptprq* in the mouse causes deafness associated with disrupted stereociliar bundles.<sup>3</sup> 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*

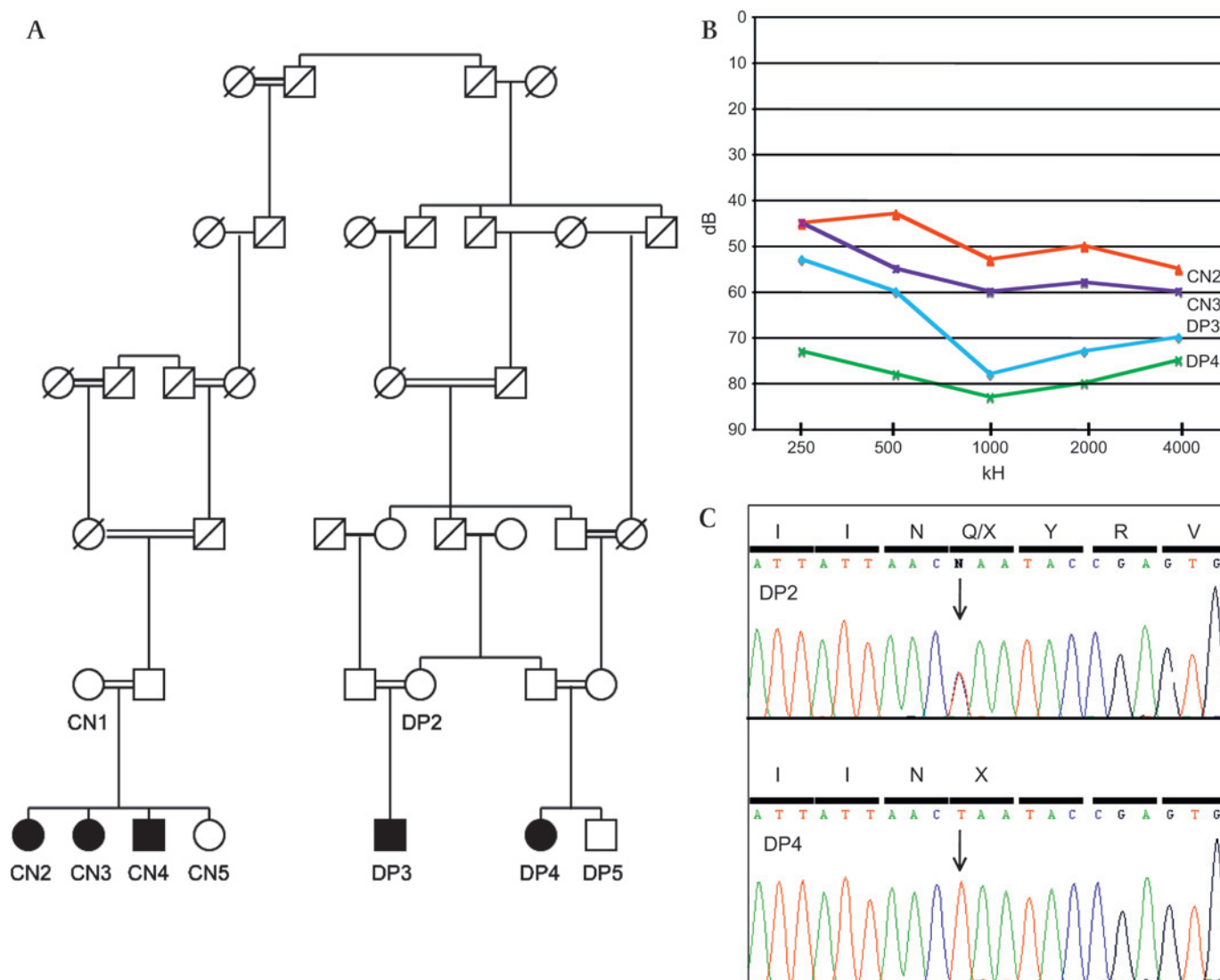
Homozygosity mapping with Affymetrix 250K single nucleotide polymorphism (SNP) arrays was performed as previously described.<sup>4</sup> Affected individuals in this study were children in two families, CN and DP. The longest deafness associated homozygous segments for these families were chr12: 74 498 486-93 289 627 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.<sup>4</sup>

### 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.<sup>5</sup> 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.<sup>6</sup>

## RESULTS

The recessive deafness locus *DFNB84* was identified by SNP based homozygosity mapping in families CN and DP.<sup>5</sup> 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.<sup>7</sup> 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.<sup>8</sup> In mouse, homozygous loss of function of *Ptprq* leads to deafness associated with absence of hair cells in the basal region of the cochlea.<sup>3</sup> *Ptprq*<sup>-/-</sup> 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*<sup>-/-</sup> mice in the context of myosin VI function.<sup>9</sup> 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.<sup>1</sup> 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.<sup>10</sup> 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.

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