Nonsense mutation of the stereociliary 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 PTPRO, 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 PTPRO 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 PTPRO appeared in any of 218 other probands with hearing loss in the family and was homozygous in all affected relatives.

Conclusion Identification of the DFNB84 gene represents the first identification of PTPRO 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 stereociliary membrane include ion channels, chemoreceptors and cell adhesion molecules. One stereociliary membrane protein is PTPRO (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 stereociliary bundles.3,4 Here we report the first human mutation of PTPRO in an extended kindred with inherited hearing loss.

METHODS
Clinical evaluation
Children with prelingual, bilateral hearing loss were ascertained through Etfaah 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.
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 PTPRO (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 PTPRO revealed c.1285C→T (at chr12:79 404 441), which is predicted to introduce stop codon TAA at codon 429, Q429X. Hearing impaired individual DP4 is homozygous for the c.1285 (p.Q429X) 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 PTPRO 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 PTPRO. Homozygous genotypes at all four markers were observed in six probands. None of these probands shared the CN/DP haplotype. Full sequencing of PTPRO 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/DF, 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 PTPRO in the ear has been assessed by evaluating Ptprq−/− mice in the context of myosin VI function.5 Myosin VI and PTPRO co-localise in the stereocilia, suggesting that the two proteins interact. In the absence of myosin VI, PTPRO 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 PTPRO 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 stereociliary membrane to the cytoskeleton. Given the conservation of these functions between humans and mice, it was to be expected that loss of function of PTPRO in humans would lead to hearing loss. The present identification of mutant PTPRO 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.

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