Nonsense mutation of the stereociliary membrane protein gene PTPRO 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 individuals in family CN/DP.

Conclusion Identification of the DFNB84 gene represents the first identification of PTPRO mutation in human 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. 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: 65 434 594-87 877 759 in family DP. The SNP haplotypes in the shared region (chr12: 74 498 486-97 775 759) were identical between families CN and DP. This locus was designated DFNB84 by the HUGO Gene Nomenclature Committee.

Mutation analysis

The human RefSeq entry for PTPRO (NM_001145026) was aligned to hg18 of the human genome. The genomic interval from Ptprq intron 5 to intron 6 is inverted on hg18. This may represent an assembly error in the reference genome rather than a true structural variant. 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.

RESULTS

The recessive deafness locus DFNB84 was identified by SNP based homozygosity mapping in families CN and DP. 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
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.

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 Otoegin, mutations in which are responsible for the mouse twister phenotype. We characterised this Otoegin-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. In mouse, homozygous loss of function of Ptprq leads to deafness associated with absence of hair cells in the basal region of the cochlea. 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. 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. 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 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 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.

**Acknowledgements** We thank the families for their participation in this study. We thank the technical staff of the Hereditary Research Laboratory of Bethlehem University, and Carlos R Gordon of Tel Aviv University/Meir General Hospital for helpful comments and suggestions. Supported by NIH/NIDCD grant R01 DC005641.

**Funding** NIH.

**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.

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*J Med Genet* 2010 47: 643-645 originally published online May 14, 2010
doi: 10.1136/jmg.2009.075697