Whole Exome Sequencing and Homozygosity Mapping Identify Mutation in the Cell Polarity Protein GPSM2 as the Cause of Nonsyndromic Hearing Loss DFNB82

Tom Walsh,1,4,* Hashem Shahin,2,4 Tal Elkan-Miller,3 Ming K. Lee,1 Anne M. Thornton,1 Wendy Roeh,1 Amal Abu Rayyan,2 Suheir Loulus,2 Karen B. Avraham,3 Mary-Claire King,1 and Moien Kanaan2,*

Massively parallel sequencing of targeted regions, exomes, and complete genomes has begun to dramatically increase the pace of discovery of genes responsible for human disorders. Here we describe how exome sequencing in conjunction with homozygosity mapping led to rapid identification of the causative allele for nonsyndromic hearing loss DFNB82 in a consanguineous Palestinian family. After filtering out worldwide and population-specific polymorphisms from the whole exome sequence, only a single deleterious mutation remained in the homozygous region linked to DFNB82. The nonsense mutation leads to an early truncation of the G protein signaling modulator GPSM2, a protein that is essential for maintenance of cell polarity and spindle orientation. In the mouse inner ear, GPSM2 is localized to apical surfaces of hair cells and supporting cells and is most highly expressed during embryonic development. Identification of GPSM2 as essential to the development of normal hearing suggests dysregulation of cell polarity as a mechanism underlying hearing loss.

Homozygosity mapping in consanguineous kindreds with nonsyndromic hearing loss has led to the discovery of more than 50 recessive deafness loci.1 Genomic regions linked to hearing loss by homozygosity mapping are generally multiple megabases in size and can contain hundreds of genes. The identification of the critical allele from a large number of candidates has heretofore been an expensive and time-consuming process. Very recently, advances in DNA enrichment and next-generation sequencing technology have made it possible to quickly and cost-effectively sequence all the genes in the “exome,” the protein-coding portion of the genome, and then to rapidly identify alleles responsible for Mendelian disorders.2,3

We applied whole exome sequencing to identify the gene responsible for nonsyndromic hearing loss DFNB82 in a Palestinian family.4 Hearing loss in affected individuals was evaluated by pure-tone audiometry and was determined to be sensorineural, prelingual, bilateral, and severe to profound. (Figure 1A). Vision and vestibular function were normal, and there were no other findings on ophthalmic testing and clinical exam. The DFNB82 region spans 3.1 Mb on chromosome 1p13.1 and contains 50 annotated protein coding genes and 1 miRNA. The DFNB82 region partially overlaps with the DFNB32 region (MIM 608653) defined by a Tunisian family with congenital profound hearing loss.5 The region of overlap contains five genes: VAV3, SLC25A4, NBPF4, NBPF6, and FAM102B.

Genomic DNA from individual CG5 (Figure 1B) was extracted from blood, sonicated to approximately 200 bp fragments, and used to make a library for paired-end sequencing (Illumina). After quality control, the library was hybridized to biotinylated cRNA oligonucleotide baits from the SureSelect Human All Exon kit (Agilent Technologies), purified by streptavidin-bound magnetic beads, amplified, and sequenced.6 The exome design covers 38 Mb of human genome corresponding to the exons and flanking intronic regions of 23,739 genes in the National Center for Biotechnology Information Consensus CDS database (September 2009 release) and also covers 700 miRNAs from the Sanger v13 database and 300 noncoding RNAs.7 We generated a total of 5.6 Gb of sequence as paired-end 76 bp reads from one lane of an Illumina Genome Analyzer IIX with Illumina pipeline v.1.6. We used MAQ v.0.7.18 to discard reads of inadequate sequence quality or depth and to discard those with identical start and end sites.

In order to identify the allele responsible for DFNB82, we focused on exonic and flanking intronic variants within the linkage region on chromosome 1p13.3 between bp 108,307,327 and 111,450,408 (February 2009 human reference sequence UCSC hg19/GRCh37). Alignment of the sequence reads in this region revealed that 424 of the 458 targeted exons (93%) had >10 high-quality reads. The fully evaluated exons include three genes (VAV3, SLC25A4, and FAM102B) from the DFNB32/DFNB82 overlap region. The other two genes in the region (NBPF4 and NBPF6) could not be evaluated because they lie on adjacent segmental duplications that are >99% identical.

In the DFNB82 region, 80 variants, all single nucleotide substitutions, passed our quality thresholds. All 80 substitutions were predicted to be homozygous, as expected given our SNP-based homozygosity mapping data.4
Of the 80 substitutions, 73 appeared in dbSNP130 and thus were not considered further and 7 were previously unidentified (Table 1). None of these seven variants were present in the sequenced exomes of three unrelated Palestinian individuals from our series. The seven variants included five synonymous substitutions, one missense substitution (MYBPHL p.I65T), and one nonsense mutation (GPSM2 p.R127X). In order to determine whether the 2 amino acid altering mutations were previously unidentified polymorphisms in the Palestinian population, we genotyped each in 192 hearing controls and 192 unrelated probands with hearing loss. The frequency of MYBPHL p.I65T was approximately 1.0% both in controls and in unrelated persons with hearing loss from this population, suggesting that it is a benign polymorphism. In contrast, GPSM2 p.R127X was not present in controls or in unrelated cases. GPSM2 p.R127X mutation segregated as expected with deafness in family CG (Figure 1A), based on PCR and Sanger sequencing of all family members.

The SNP databases include two entries (rs36018922 and rs34430749) in exon 4 of GPSM2 that are predicted to lead to premature truncations. However, each of these variants was reported only once, neither was validated by the reporting source, and we did not observe either in 768 unrelated individuals (384 Palestinians, 192 Caucasians, 192 African Americans, data not shown). These entries were likely artifacts of alignment errors in short mononucleotide runs.

GPSM2, also known as LGN (Leu-Gly-Asn repeat-enriched protein) and Pins (human homolog of Drosophila, Partner of Inscuteable), is a 677 amino acid, G protein signaling modulator. It is comprised of seven N-terminal tetratricopeptide (TPR) motifs, a linker domain, and four C-terminal Ga_i/o-GoLoco motifs (GoLoco) motifs that function in guanine nucleotide exchange. The DFNB82 mutation is predicted to truncate the protein between the second and third TPR motifs (Figure 1D). Analysis of lymphoblast RNA from CG14, a normal hearing parent and heterozygous carrier of
p.R127X, indicates that the nonsense allele produces a stable mutant transcript (see Figure S1 available online).

**GPSM2** is widely expressed, but its pattern in the ear has not previously been described. We evaluated inner ear sections from embryonic day 16.5 (e16.5), postnatal day 0 (p0), p15, and p60 mice by immunohistochemistry with an antibody against Gpsm2\(^2\) (ProteinTech) (Figure 2; Figure S2). Staining indicates localization at the apical surfaces of the hair and supporting cells in the cochlea (Figures 2C, 2D, 2G, and 2H), utricle (Figures 2F and 2J), and saccule and cristae (data not shown) of e16.5 and p0 inner ears. In the cochlea, GPSM2 localization extends throughout the greater epithelial ridge (Figures 2E and 2I). Cochlear hair cells at p0 exhibit an asymmetrical localization of Gpsm2 at the lateral edge, a pattern that is not present in the e16.5 hair cells. Gpsm2 is also localized in the pillar cells in p0 inner ears. By p15, Gpsm2 disappears at the apical surface of the cells, whereas it persists in pillar cells. In adult mice (p60), Gpsm2 is concentrated specifically in the head region of the inner pillar cells (Figure S2).

In order to characterize expression of Gpsm2 during development, we assessed levels of Gpsm2 transcript expression in the mouse ear by quantitative RT-PCR of RNA from wild-type C3H mice at ages e16.5, p0, p7, p30, and p90 (Figure 3). Relative to levels of the internal control Hprt, Gpsm2 expression decreased rapidly between e16.5 and e30 to a level in adult mice approximately 20% of the level at birth.

Pins, the *Drosophila* homolog of GPSM2, has been well characterized. Pins is an evolutionarily conserved protein.

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**Table 1. Private Variants in Genes in the DFNB82 Region**

<table>
<thead>
<tr>
<th>Genomic Site (hg19)</th>
<th>Variant</th>
<th>Gene</th>
<th>Amino Acid Effect</th>
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<tr>
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<td>AKNAD1</td>
<td>H806H</td>
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<td>chr1: 109,394,540 G&gt;A</td>
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<td>Y250Y</td>
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<tr>
<td>chr1: 109,440,214 C&gt;T</td>
<td>GPSM2</td>
<td>R127X</td>
<td>Nonsense; not present in Palestinian controls</td>
</tr>
<tr>
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<td>KIAA1324</td>
<td>A61A</td>
<td>Silent</td>
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<tr>
<td>chr1: 109,840,834 A&gt;G</td>
<td>MYBPHL</td>
<td>I65T</td>
<td>Missense; polymorphic in Palestinian controls</td>
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<tr>
<td>chr1: 110,085,767 G&gt;A</td>
<td>GPR61</td>
<td>S42S</td>
<td>Silent</td>
</tr>
</tbody>
</table>

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Figure 2. Localization of Gpsm2 in the Mouse Inner Ear

(A and B) Schematic representation of the cochlea (A) and utricle (B), one of the vestibular organs. The inner and outer hair cells of the cochlea make up the sensory cells; they are embedded in non-sensory supporting cells, including the Deiters, pillar, and Hensen cells. The utricle also contains hair cells and supporting cells. (C–J) Immunofluorescence images of the cochlea at e16.5 (C and D) and p0 (G and H) and of the utricle at e16.5 (F) and p0 (J). Gpsm2 (green) is localized at the apical surface of the hair and supporting cells, both in the cochlea and utricle and along the greater epithelial ridge of the hair cell surface. It is also localized in the pillar cells at this age. Hair cell cytoplasm is marked by myosin VI (red), and the nuclei are marked by DAPI (blue). Paraffin embedded sections were stained with primary antibodies goat anti-myosin VI (Santa Cruz Biotechnology) and rabbit anti-Gpsm2 (ProteinTech; for validation of the antibody, see 12) and the fluorescence-conjugated secondary antibodies donkey anti-rabbit 488 (Molecular Probes) and donkey anti-goat cy3 (Sigma). Imaging was done with an LSM 510 confocal microscope (Zeiss). Scale bars represent 5 μm (C, E–G, I, and J), 2 μm (D), and 2.5 μm (H). All procedures involving animals met NIH guidelines and were approved by the Animal Care and Use Committees of Tel Aviv University and the University of Washington.
In *Drosophila* neuroblasts, Pins regulates apical-basal spindle orientation and asymmetric cell division. In *Caenorhabditis elegans* one-cell embryos, the Pins functional counterparts GPR-1 and GPR-2 are essential for translating polarity cues into asymmetric spindle positioning. Mammals have two Pins homologs, GPSM1 and GPSM2. GPSM1 expression is tissue specific, primarily being enriched in the brain, testis, and heart. GPSM1 maps to a locus associated with recessive nonsyndromic deafness (*DFNB79*, MIM 613307) but has recently been excluded as the cause of this hearing loss. GPSM2 appears to be ubiquitously expressed.

A knockout mouse model of Gpsm2 has recently been generated with premature truncation in the C-terminal GoLoco motifs but with the TPR repeats predicted to be intact. Mice homozygous for this truncation (designated LGN<sup>Dc/Dc</sup>) are viable and fertile. No additional phenotyping or behavioral studies were described for these mice. At the cellular level, mitotic orientations within the apical divisions of the dorsolateral brain of the LGN<sup>Dc/Dc</sup> mice were random, suggesting that GPSM2 is essential for planar spindle orientation during apical divisions.

The establishment and maintenance of cell polarity involves coordinated regulation of signaling cascades, membrane trafficking, and cytoskeletal dynamics. Dysregulation of cell polarity can lead to developmental disorders. GPSM2, along with G protein alpha and the nuclear mitotic apparatus protein NuMA, is a major contributor in establishing and maintaining cellular polarity in yeast, worms, flies, and mammals.

In the inner ear, premature truncation of GPSM2 may be associated with defects in cell polarity, which relies on asymmetric organization of cellular components, scaffolding molecules, and structures along the apical-basal cellular axis. Hair and sensory epithelial cells of the organ of Corti have polarized structures and functions. Hair cells have a defined apical localization of the stereocilia that form a hair bundle polarized along their planar axis. The apical underlying network of actins and the planar polarity of hair bundles endow hair cells with coordinated directional response to mechanical signals. These polarities are examples of cellular differentiation with asymmetric distribution of molecules that regulate apical-basal polarity and planar cell polarity. GPSM2, G protein alpha, and NuMA are associated with the apical cortical crescent. Gpsm2 is localized along the apical surface of the organ of Corti and vestibular organs at e16.5 and p0 in the mouse. By p0, its localization in hair cells is limited to the lateral edge. Such a pattern is characteristic of proteins of the planar cell polarity pathway, such as Vangl2, Dvl2, and Fz3, where the protein is asymmetrically localized to one edge of the apical surface of the cells. Like Gpsm2, Wnt7a, shown to mediate stereociliary bundle reorientation, becomes restricted to the pillar cells with age. Pillar cells have been proposed to act as the organizing center for the orientation of the organ of Corti. We speculate that the establishment of polarity during cellular differentiation and the maintenance of proper polarity requires GPSM2 and that loss of GPSM2 function by premature truncation of the GPSM2 protein would lead to abnormal orientation of hair bundles and hence to aberrant hair cell transduction and hearing loss.

**Supplemental Data**

Supplemental Data include two figures and can be found with this article online at [http://www.ajhg.org](http://www.ajhg.org).

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**Web Resources**

The URLs for data presented herein are as follows:

References


