Published in final edited form as:

Hear Res. 2008 March ; 237(1-2): 57–65.

Expression studies of osteoglycin/mimecan (*OGN*) in the cochlea and auditory phenotype of *Ogn*-deficient mice

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Abstract

Genes involved in the hearing process have been identified through both positional cloning efforts following genetic linkage studies of families with heritable deafness and by candidate gene approaches based on known functional properties or inner ear expression. The latter method of gene discovery may employ a tissue- or organ-specific approach. Through characterization of a human fetal cochlear cDNA library, we have identified transcripts that are preferentially and/or highly expressed in the cochlea. High expression in the cochlea may be suggestive of a fundamental role for a transcript in the auditory system. Herein we report the identification and characterization of a transcript from the cochlear cDNA library with abundant cochlear expression and unknown function that was subsequently determined to represent osteoglycin (*OGN*). *Ogn*-deficient mice, when analyzed by auditory brainstem response and distortion product otoacoustic emissions, have normal hearing thresholds.

Keywords

mimecan/osteoglycin; proteoglycan; hearing loss

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INTRODUCTION

Hearing loss is the most common sensory deficit in humans (reviewed in (Morton et al., 2006)). At least half of these cases are due to genetic causes (Toriello et al., 2004); more than 130 loci have been identified and more than 65 genes have been cloned to date ((<http://webhost.ua.ac.be/hhh>), accessed December 2007). A large variety of gene families are involved in hearing loss, including gap junction proteins, ion channels, and collagens, illustrating the great biomolecular complexity of the auditory system. Even with the tremendous successes in gene discovery in hearing in the last few years, there are still numerous loci for which genes have yet to be identified, and many functional processes of the inner ear which have not yet been elucidated. To focus investigations in understanding the biology of hearing on a molecular level, it can be fruitful to study those genes highly expressed in the inner ear.

To facilitate the search for genes potentially important in hearing and deafness, a human fetal cochlear cDNA library was constructed and analyzed using subtractive techniques to isolate transcripts preferentially expressed in the cochlea (Robertson et al., 1994). cDNA clones were sequenced and resulting expressed sequence tags (ESTs) analyzed and deposited in GenBank (www.ncbi.nlm.nih.gov) (Skvorak et al., 1999; Resendes et al., 2002). EST cluster analysis was performed to identify highly expressed clones and to prioritize cochlear clones for additional studies. Several auditory genes, namely *ATQ1*, *COCH*, *OTOR* and *PFET1*, of which the latter three are preferentially expressed in cochlea, have been identified using this library (Robertson et al., 1997; Robertson et al., 2000; Skvorak et al., 1997; Resendes et al., 2004). *COCH* has since been shown to be mutated in DFNA9, a dominantly inherited sensorineural deafness and vestibular disorder (Robertson et al., 1998). Examination of UniGene dbEST and NCBI Blast searches suggests that *OTOR* is an inner ear-specific gene (Beisel et al., 2004) and under human-specific selection (Clark et al., 2003).

As a result of the EST cluster analysis, we determined that EST H88966 represented a cDNA clone abundantly expressed in cochlear tissue. Subsequent sequence analysis revealed that this cDNA encoded osteoglycin (*OGN*). *OGN* is a member of a class of proteoglycans (PGs) called small leucine-rich proteoglycans (SLRPs) residing in the extracellular matrix of connective tissues and involved in matrix assembly, cellular growth and migration. *OGN* is one of the three major keratan sulfate-containing PGs in the cornea. SLRPs can be modified with various glycosaminoglycan (GAG) side chains that appear to affect fibril diameter, interfibrillar spacing and normal tissue hydration and, in the cornea, the keratan sulfate GAGs are believed thereby to function in corneal transparency (Funderburgh et al., 1997). To study the gene's function further, an *Ogn*-deficient mouse was created (Tasheva et al., 2002). Corneal development and clarity appear normal in *Ogn*-deficient mice, but it was reported initially by transmission electron microscopy that the diameter of the corneal collagen fibrils in these mice was significantly increased although this was not confirmed subsequently by X-ray diffraction studies (Beecher et al., 2005). This increase was also reported initially in the collagen fibrils of the skin. In view of the abundant expression of *OGN* in the cochlea and its potential role in regulating the thickness of collagen fibrils in eye and skin, and the fundamental role of collagens in hearing, we explored the hearing status of *Ogn*-deficient mice.

MATERIALS AND METHODS

Human Northern Blot Analysis

Total cellular RNAs were extracted (Chirgwin et al., 1979) from second trimester human fetal organs including thymus, cochlea (membranous labyrinths), spleen, lung, tongue, testis, liver, kidney, bone marrow, and skeletal muscle. All human tissues were obtained in accordance with guidelines established by the Partners HealthCare System Human Research Committee. Ten

micrograms of each of the RNAs were electrophoresed in a denaturing 1% agarose-formaldehyde gel and transferred to GeneScreen filters (DuPont, Wilmington, DE). Ethidium bromide-stained RNAs were visualized after electrophoresis to confirm integrity and concentration of RNAs. Filters were prehybridized for 2–4 h and hybridized overnight at 42°C with a ³²P-labeled H88966 probe in 10% dextran sulfate, 4X SSC, 7 mM Tris-HCl (pH 7.6), 0.8X Denhardt's solution, and 20 ug/ml sonicated herring sperm DNA in 40% formamide and 0.5% SDS. EST H88966 sequence corresponds to the 3' end of the 3'UTR of *OGN*, spanning nucleotides 2597–2960 of NM_033014 (<http://www.ncbi.nlm.nih.gov/Genbank>). Filters were washed in 0.1X SSC in 0.1% SDS at 42–55°C prior to autoradiography using XAR-5 film with intensifying screens at –80°C. Control hybridizations of these filters with a *GAPDH* (NM_002046.3) probe verified equal loading and integrity of the transferred RNAs and were consistent with semi-quantitative estimates of RNA concentrations as visualized by ethidium bromide staining of the gel.

Human Inner Ear cDNA Microarray Analysis

Over 4,500 cDNAs from the Morton human fetal cochlear cDNA library were PCR amplified, purified and spotted in duplicate on a human inner ear cDNA microarray. The array also includes positive and negative control cDNAs, and cDNAs for genes *CDH23*, *GJB3*, *KVLQT1*, *MYO7A*, *MYO15A*, *PCDH15*, *SLC26A4*, *USH2A* and *WFS1* involved in human hearing loss not present among cDNA clones identified in an inner ear EST collection. Total human fetal cochlear RNA and a reference RNA (Universal Human Reference (UHR), Stratagene, La Jolla, CA) were labeled with fluorescent dyes Cy-3 or Cy-5 by both direct labeling and indirect labeling techniques (two separate experiments, three slides each, including dye swaps) and hybridized to the microarrays using the Pronto Plus microarray system (Promega, Madison, WI) according to manufacturers' protocols. Hybridized slides were scanned to measure relative fluorescence intensity of each cDNA spot. Raw data from the relative ratios of gene expression in the two samples were filtered and normalized. Only genes with consistent expression across all six slides were considered positives. Each positive cDNA from the array was sequence verified.

Mouse Expression Analysis

Sajan et al. recently completed an analysis of gene expression in the developing mouse inner ear and non-inner ear tissues (Sajan et al., 2007). The inner ear tissues included in each of the stages were distributed as follows: inner ear epithelia (E9-E10); ventral cochlear tissue samples distinct from dorsal vestibular tissue samples (E10.5-E12); and cochlear tissue samples dissected from the saccule samples separated from a combined sample of the utricles and ampullae (E12.5-E15). The non-inner ear samples included: neuroepithelial and mesenchymal cells (E9); ganglia and mesenchymal and vascular cells (E9.5- E10.5); and mesenchyme, vascular, the modiolus and early cartilage (E11-E15). The Affymetrix mouse MOE430A_2.0 gene chip was used as described to assess expression of 13,000–14,000 unique genes at half-day intervals from E9-E15. Two different probe sets were identified which represented the *Ogn* transcript: 1419662_at, which spans 3 exons of *Ogn*, and 1419663_at, which is located at the end of the 3' UTR.

A second experiment was performed in which gene expression in the developing mouse utricle, from stages E10.5 to 17 months, was analyzed on the Affymetrix mouse MOE430 2.0 chip. Utricles were collected at daily intervals and replicates were used for hybridization at each stage. dChip software (Li et al., 2001) was used to analyze the data. The same two probe sets mentioned above also served to measure *Ogn* expression in these experiments.

Ogn-Deficient Mice

Ogn^{-/-} mice were created in a mixed background of C57BL/6 and 129/Ola as described (Tasheva et al., 2002). Briefly, exon 2 containing the start codon was interrupted with a neo cassette. Genomic replacement was demonstrated through Southern blot analysis and loss of transcript was verified through RT-PCR and Northern blot analysis using the *Ogn* cDNA as a probe. Experimentation on animals was approved by Harvard University's Animal Care and Use Committee. To obtain a more homogeneous genetic background, *Ogn*^{+/-} were outcrossed to CBA/CaJ wild-type mice for three generations and resultant heterozygote offspring were intercrossed to obtain new *Ogn*-deficient, heterozygote, and wild-type mice. Mice of the CBA/CaJ strain were selected for breeding due to their recognized "good" hearing status among inbred strains. *Ogn*^{-/-} mice in the CBA/CaJ background were analyzed for hearing status at 6 weeks and again at 6 months.

Auditory Brainstem Response

Auditory brainstem responses (ABRs) were measured bilaterally in all *Ogn* genotypes. Mice were anesthetized with xylazine (20 mg/kg ip) and ketamine (100 mg/kg ip) and electrodes were inserted at the vertex and pinna, with a ground near the tail. ABR potentials were evoked with 5-ms tone pips (0.5-ms rise-fall with a cos² onset envelope, delivered at 35/s). The response was amplified (10,000x), filtered (100 Hz-3 kHz), and averaged with an A-D board in a LabVIEW-driven data-acquisition system. Sound level was increased in 5 dB steps from 10 dB below threshold to ≤80 dB SPL. At each sound level, 1,024 responses were averaged (with stimulus polarity alternated) using an "artifact-reject", whereby response waveforms were discarded when peak-to-peak amplitude exceeded 15 μV. On visual inspection of stacked waveforms, "threshold" was defined as the lowest SPL level at which any wave could be detected, usually corresponding to the level step just below that at which the peak-peak response amplitude rose significantly above the noise floor (approximately 0.25 μV). For amplitude versus level functions, the wave I peak was identified by visual inspection at each sound level and the peak-to-peak amplitude computed.

Distortion Product Otoacoustic Emission Responses

Animals were anesthetized as described for ABR testing. Distortion product otoacoustic emissions (DPOAEs) at 2 f_1 - f_2 were recorded with a custom acoustic assembly consisting of two 0.25 inch condenser microphones to generate primary tones (f_1 and f_2 with $f_2/f_1 = 1.2$ and f_2 level at 10 dB < f_1 level) and Knowles miniature microphone (EK3103) to record ear-canal sound pressure. Stimuli were generated digitally (AO-6, National Instruments, Austin, TX). Ear canal sound pressure was amplified and digitally sampled at 20 ms (A-2000, National Instruments). Fast Fourier Transforms were computed and averaged over five consecutive waveform traces, and 2 f_1 - f_2 DPOAE amplitude and surrounding noise floor were extracted, a procedure requiring approximately 4 s of data acquisition and processing time.

RESULTS

Human Expression Analysis

Initial sequence analysis of the cochlear cDNA library identified 12 ESTs representing osteoglycin. To assess osteoglycin transcript level and tissue distribution, Northern blot analysis was performed using RNAs prepared from human fetal organs including brain, thymus, cochlea, spleen, lung, tongue, testis, liver, kidney, bone marrow, and skeletal muscle (Fig. 1). *OGN* expression was detected in several tissues, with the most abundant expression observed in cochlea.

A high level of *OGN* expression was also detected on microarrays spotted with the cochlear cDNA library. Eighty spots showed differential expression across all six slides: 42 genes had higher expression in cochlea and 38 genes had higher expression in the reference sample. Each positive cDNA from the array was sequence verified. The 10 genes showing the highest expression in cochlea relative to the reference RNA are listed in Table 1.

OGN was found to be expressed 10-fold higher in human cochlear RNA than in Universal Human Reference (UHR) RNA. This abundant cochlear expression is in agreement with data obtained from the Expression Profile of osteoglycin found in UniGene (www.ncbi.nlm.nih.gov/UniGene). Out of a pool of 16,942 ESTs from ear tissue, 95 of them encode *OGN*, standardized to 5607/10⁶. In comparison, the next two most *OGN*-rich tissues were vascular tissue (1894/10⁶) and parathyroid (378/10⁶). Data from UniGene also suggest that *OGN* may have a significant role in development with a greater than six-fold increase in embryonic tissue--242/10⁶ transcripts in embryonic tissue, but only 38/10⁶ in adult tissue.

Mouse Expression Analysis

Analysis of the results of Sajan et al. (2007) revealed that *Ogn* expression in the mouse inner ear tissues increased through the developmental time points from E9 through to E15 (Fig. 2). A search for other genes which shared a similar pattern of inner ear expression suggested a correlation with *Atoh1*, also known as *Math1*, with a Pearson correlation coefficient of 0.78. The expression of the two genes in the non-inner ear samples was not at all correlated. *Atoh1* had low level expression at all three time points (with an absent value at E9, a value of 71.1 +/- 12.5 at E9.5-E10.5, and a value of 23.1 +/- 9.3 at E11-E15) while *Ogn* expression increased sharply from E9.5-E15 (with absent values at both E9 and E9.5-E10.5 and increasing to a value of 312.7 +/- 26.4 at E11-E15).

An independent analysis of gene expression in the developing mouse utricle was done to establish the expression of *Ogn* from E10.5 to P12. This study also showed that *Ogn* expression increased from E10.5 to birth. With the inclusion of postnatal time points, it was also possible to determine that *Ogn* remained expressed in the utricle after birth. A list of extracellular matrix genes that share a similar expression pattern is included in Table 2.

Cochlear Function

Ogn^{-/-} mice were originally created in a mixed genetic background of C57BL/6 and 129/Ola. In an effort to minimize phenotypic variability, *Ogn*^{+/-} males were out-crossed to wild-type CBA/CaJ females for three generations. Three *Ogn*^{-/-} mice, four *Ogn*^{+/-} and five *Ogn*^{+/+} mice underwent ABR and DPOAE testing at six weeks of age to assess auditory function. At this age, the cochlea is fully developed and early-onset progressive hearing loss present in many inbred strains has not yet begun to confound the analysis. No significant threshold differences among genotypes were observed at this time point (p=0.306 by 2-way ANOVA). These mice were tested again at 6 months of age. Mean DPOAE thresholds were slightly better at high frequencies in *Ogn*^{-/-} (when compared to wildtype), however, the difference failed to reach significance at the p=0.01 level. Suprathreshold amplitudes for DPOAEs and ABRs were also examined, and no significant effects of *Ogn* deficiency were observed (Fig. 3).

DISCUSSION

Identification of genes highly expressed in the cochlea has served as a powerful means of gene discovery in the auditory system (Robertson et al., 1997; Robertson et al., 2000; Skvorak et al., 1997; Resendes et al., 2004), including genes subsequently determined to cause human hearing impairments (e.g., *COCH* (Robertson et al., 1998), *CRYM* (Abe et al., 2003)). Here, we studied a gene that was found to be expressed abundantly in the human fetal cochlear cDNA

library by EST, Northern blot and microarray analysis. EST analysis showed that EST H88966 was a member of a cluster of 12 ESTs and represented an abundantly expressed cochlear transcript. Sequence analysis showed that this EST represented the known gene, *OGN*, encoding osteoglycin, which maps to 9q22. Northern blot analysis demonstrated that the transcript was expressed in several tissue types with highest expression in the cochlea. Further evidence for the importance of *OGN* in the auditory system was supported by the analysis of RNA abundance using an inner ear cDNA microarray. When the hybridization pattern of human fetal cochlear RNA was compared to that of control RNA, *OGN* was the tenth most abundant transcript among clones present on the inner ear array. The finding that *OGN* is present at relatively high levels of expression in the cochlea suggests a fundamental role in the biology of the inner ear.

Osteoglycin is a small leucine-rich proteoglycan (SLRP) with a role in collagen fibrillogenesis in eye and skin (Ge et al., 2004; Tasheva et al., 2002). This observation suggests potential involvement for osteoglycin with the abundant collagens present in the inner ear. Various types of collagen are needed to maintain the precise structures of the cochlea. The fundamental importance of these collagens is demonstrated by the impressive number of hearing disorders caused by their mutation: *COL4A5*, *COL4A3*, *COL4A4* in Alport Syndrome; *COL2A1*, *COL11A1*, *COL11A2*, *COL9A1* in Stickler syndrome; and *COL11A2* in DFNA13 (Vikkula et al., 1995; McGuirt et al., 1999; Renieri et al., 1992a; Renieri et al., 1992b; Smeets et al., 1992; Lemmink et al., 1994b; Lemmink et al., 1994a; Mochizuki et al., 1994; Ahmad et al., 1991; Van Camp et al., 2006). If osteoglycin participates in the proper development of these proteins, it is reasonable to hypothesize that a deficiency of *OGN* would potentially lead to disruption of the structural integrity of the inner ear and, thereby, affect normal hearing. As assessed at the time of submission, no loci for any human deafness disorders have been mapped to the region containing *OGN* (<http://webhost.ua.ac.be/hhh/>), but many genes remain to be identified for >400 forms of syndromic and 120 forms of nonsyndromic deafnesses.

Mouse expression studies were performed to establish that *Ogn* was increasingly expressed during the development of the inner ear. An initial study evaluating time points from E9-E15 noted a similar expression pattern of *Atoh1*. *Atoh1* is a basic helix-loop-helix transcription factor which has been found to be a crucial gene for inner ear development. Mice deficient for *Atoh1* show a complete loss of hair cells, while maintaining a normal overall structure of the inner ear (Bermingham et al., 1999). The correlated expression of *Atoh1* and *Ogn* is of interest, and possibly suggests that *Ogn* has a role in hair cell development. Additionally, Sajan et al. (2007) note that the genes they identified to be up-regulated in the non-inner ear tissues were predominantly involved in the extracellular matrix or in the cytoskeleton (Sajan et al., 2007). Due to the role of osteoglycin in collagen fibrillogenesis, *Ogn* fits into this category and provides additional support for a structural role in the developing auditory system.

Additional expression studies in the mouse utricle from E10 through to P12 revealed that the expression of *Ogn* in the mouse utricle was correlated with that of a number of other genes (Table 2). Notably, a number of collagen genes, including *COL1A1*, *COL1A2*, *COL4A3*, *COL4A5*, *COL11A1* and *COL11A2* are in this list. Mutations in *COL1A1* and *COL1A2* are responsible for osteogenesis imperfecta (Pope et al., 1985; Pihlajaniemi et al., 1984) and *COL1A2* is also one of the most highly expressed in the Morton cochlear cDNA library (Skvorak et al., 1999; Resendes et al., 2002). *COL4A3* and *COL4A5* mutations are associated with Alport syndrome (Lemmink et al., 1994b; Barker et al., 1990), while disruption of *COL11A1* and *COL11A2* causes Stickler syndrome (Richards et al., 1996; Vikkula et al., 1995). Several modifiers of extracellular matrix, including matrix metalloproteinase 2 (Mmp2), Mmp14 and Mmp15 also share a similar expression pattern. The correlated expression of *Ogn* with these genes is further evidence that *Ogn* might play a role in regulating the collagen structures of the auditory system.

Though the absence of a hearing loss phenotype in *Ogn*^{-/-} mice suggests that the gene may not be critical in the auditory system of the mouse, the gene may be important in the human hearing process. Mouse models may replicate the corresponding human phenotype, but this is certainly not always the case. *OGN* may be involved in hearing loss in a mutation specific manner such that a null mouse model would not mimic a human phenotype. One prime example from the hearing literature is that of *COCH* and its role in DFNA9. *COCH* was chosen as a candidate for hearing loss due to its high expression in the Morton fetal cochlear cDNA library (Robertson et al., 1994; Robertson et al., 1997). Mutations in the gene have since been found to be responsible for DFNA9 in a number of families around the world (Robertson et al., 1998; de Kok et al., 1999; Fransen et al., 1999; Kamarinos et al., 2001; Usami et al., 2003; Nagy et al., 2004; Kemperman et al., 2005; Street et al., 2005; Collin et al., 2006; Pauw et al., 2007). But, although this relationship is well-established, mice deficient in *Coch* do not exhibit hearing loss (Makishima et al., 2005). This discrepancy may be because of the differences in the physiology between mouse and human or may be due to the specific mutations causing a gain-of-function effect that will only be observed from knock-in studies.

Likewise, *OGN* may be involved in hearing loss, but its effect may not be significant in the mice studied. In addition, it is possible that other members of the SLRP family are able to compensate for the loss of a family member as demonstrated by the lack of a vision phenotype in these *Ogn*^{-/-} mice (Tasheva et al., 2002) and the subtle phenotype observed in fibromodulin-null mice (Svensson et al., 1999).

Acknowledgements

We thank Bradley J. Quade, M.D., Ph.D. for assistance in developing the inner ear cDNA microarray. We also thank Weijia Zhang, Ph.D. for assistance in analyzing the inner ear cDNA microarray. Special thanks to Samin Sajjan for his assistance with identifying other genes in the inner ear with expression patterns which correlate with that of osteoglycin. This research was supported by NIH grants F31 DC005712 (to R.E.W.), F32 DC00405 (to B.L.R.), R01 DC04546 and R01 DC06908 (to Z.-Y.C.), R01 DC0188 and P30 DC5209 (to M.C.L.), R01 DC03402 and P01 GM061354 (to C.C.M.) and EY13395 (to G.W.C and E.S.T). M.H. was supported in part by a grant from the Deafness Research Foundation.

References

- Abe S, Katagiri T, Saito-Hisaminato A, Usami S, Inoue Y, Tsunoda T, Nakamura Y. Identification of CRYM as a candidate responsible for nonsyndromic deafness, through cDNA microarray analysis of human cochlear and vestibular tissues. *Am J Hum Genet* 2003;72:73–82. [PubMed: 12471561]
- Ahmad NN, Ala-Kokko L, Knowlton RG, Jimenez SA, Weaver EJ, Maguire JJ, Tasman W, Prockop DJ. Stop codon in the procollagen II gene (COL2A1) in a family with the Stickler syndrome (arthro-ophthalmopathy). *Proc Natl Acad Sci U S A* 1991;88:6624–7. [PubMed: 1677770]
- Barker DF, Hostikka SL, Zhou J, Chow LT, Oliphant AR, Gerken SC, Gregory MC, Skolnick MH, Atkin CL, Tryggvason K. Identification of mutations in the COL4A5 collagen gene in Alport syndrome. *Science* 1990;248:1224–7. [PubMed: 2349482]
- Beecher N, Carlson C, Allen BR, Kipchumba R, Conrad GW, Meek KM, Quantock AJ. An x-ray diffraction study of corneal structure in mimecan-deficient mice. *Invest Ophthalmol Vis Sci* 2005;46:4046–9. [PubMed: 16249479]
- Beisel KW, Shiraki T, Morris KA, Pompeia C, Kachar B, Arakawa T, Bono H, Kawai J, Hayashizaki Y, Carninci P. Identification of unique transcripts from a mouse full-length, subtracted inner ear cDNA library. *Genomics* 2004;83:1012–23. [PubMed: 15177555]
- Bermingham NA, Hassan BA, Price SD, Vollrath MA, Ben-Arie N, Eatock RA, Bellen HJ, Lysakowski A, Zoghbi HY. *Math1*: an essential gene for the generation of inner ear hair cells. *Science* 1999;284:1837–41. [PubMed: 10364557]
- Chirgwin JM, Przybyla AE, MacDonald RJ, Rutter WJ. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* 1979;18:5294–9. [PubMed: 518835]
- Clark AG, Glanowski S, Nielsen R, Thomas PD, Kejariwal A, Todd MA, Tanenbaum DM, Civello D, Lu F, Murphy B, Ferreira S, Wang G, Zheng X, White TJ, Sninsky JJ, Adams MD, Cargill M. Inferring

- nonneutral evolution from human-chimp-mouse orthologous gene trios. *Science* 2003;302:1960–3. [PubMed: 14671302]
- Collin RW, Pauw RJ, Schoots J, Huygen PL, Hoefsloot LH, Cremers CW, Kremer H. Identification of a novel COCH mutation, G87W, causing autosomal dominant hearing impairment (DFNA9). *Am J Med Genet A* 2006;140:1791–4. [PubMed: 16835921]
- de Kok YJ, Bom SJ, Brunt TM, Kemperman MH, van Beusekom E, van der Velde-Visser SD, Robertson NG, Morton CC, Huygen PL, Verhagen WI, Brunner HG, Cremers CW, Cremers FP. A Pro51Ser mutation in the COCH gene is associated with late onset autosomal dominant progressive sensorineural hearing loss with vestibular defects. *Hum Mol Genet* 1999;8:361–6. [PubMed: 9931344]
- Fransen E, Verstreken M, Verhagen WI, Wuyts FL, Huygen PL, D’Haese P, Robertson NG, Morton CC, McGuirt WT, Smith RJ, Declau F, Van de Heyning PH, Van Camp G. High prevalence of symptoms of Meniere’s disease in three families with a mutation in the COCH gene. *Hum Mol Genet* 1999;8:1425–9. [PubMed: 10400989]
- Funderburgh JL, Corpuz LM, Roth MR, Funderburgh ML, Tasheva ES, Conrad GW. Mimecan, the 25-kDa corneal keratan sulfate proteoglycan, is a product of the gene producing osteoglycin. *J Biol Chem* 1997;272:28089–95. [PubMed: 9346963]
- Ge G, Seo NS, Liang X, Hopkins DR, Hook M, Greenspan DS. Bone morphogenetic protein-1/tolloid-related metalloproteinases process osteoglycin and enhance its ability to regulate collagen fibrillogenesis. *J Biol Chem* 2004;279:41626–33. [PubMed: 15292192]
- Kamarinos M, McGill J, Lynch M, Dahl H. Identification of a novel COCH mutation, I109N, highlights the similar clinical features observed in DFNA9 families. *Hum Mutat* 2001;17:351. [PubMed: 11295836]
- Kemperman MH, De Leenheer EM, Huygen PL, van Duijnhoven G, Morton CC, Robertson NG, Cremers FP, Kremer H, Cremers CW. Audiometric, vestibular, and genetic aspects of a DFNA9 family with a G88E COCH mutation. *Otol Neurotol* 2005;26:926–33. [PubMed: 16151339]
- Lemmink HH, Kluijtmans LA, Brunner HG, Schroder CH, Knebelmann B, Jelinkova E, van Oost BA, Monnens LA, Smeets HJ. Aberrant splicing of the COL4A5 gene in patients with Alport syndrome. *Hum Mol Genet* 1994a;3:317–22. [PubMed: 8004101]
- Lemmink HH, Mochizuki T, van den Heuvel LP, Schroder CH, Barrientos A, Monnens LA, van Oost BA, Brunner HG, Reeders ST, Smeets HJ. Mutations in the type IV collagen alpha 3 (COL4A3) gene in autosomal recessive Alport syndrome. *Hum Mol Genet* 1994b;3:1269–73. [PubMed: 7987301]
- Li J, Wong L. Emerging patterns and gene expression data. *Genome Inform* 2001;12:3–13. [PubMed: 11791219]
- Makishima T, Rodriguez CI, Robertson NG, Morton CC, Stewart CL, Griffith AJ. Targeted disruption of mouse Coch provides functional evidence that DFNA9 hearing loss is not a COCH haploinsufficiency disorder. *Hum Genet* 2005;118:29–34. [PubMed: 16078052]
- McGuirt WT, Prasad SD, Griffith AJ, Kunst HP, Green GE, Shpargel KB, Runge C, Huybrechts C, Mueller RF, Lynch E, King MC, Brunner HG, Cremers CW, Takanosu M, Li SW, Arita M, Mayne R, Prockop DJ, Van Camp G, Smith RJ. Mutations in COL11A2 cause non-syndromic hearing loss (DFNA13). *Nat Genet* 1999;23:413–9. [PubMed: 10581026]
- Mochizuki T, Lemmink HH, Mariyama M, Antignac C, Gubler MC, Pirson Y, Verellen-Dumoulin C, Chan B, Schroder CH, Smeets HJ, et al. Identification of mutations in the alpha 3(IV) and alpha 4 (IV) collagen genes in autosomal recessive Alport syndrome. *Nat Genet* 1994;8:77–81. [PubMed: 7987396]
- Morton CC, Nance WE. Newborn hearing screening--a silent revolution. *N Engl J Med* 2006;354:2151–64. [PubMed: 16707752]
- Nagy I, Horvath M, Trexler M, Repassy G, Patthy L. A novel COCH mutation, V104del, impairs folding of the LCCL domain of cochlin and causes progressive hearing loss. *J Med Genet* 2004;41:e9. [PubMed: 14729849]
- Pauw RJ, Huygen PL, Collin RW, Cruysberg JR, Hoefsloot LH, Kremer H, Cremers CW. Phenotype description of a novel DFNA9/COCH mutation, I109T. *Ann Otol Rhinol Laryngol* 2007;116:349–57. [PubMed: 17561763]

- Pihlajaniemi T, Dickson LA, Pope FM, Korhonen VR, Nicholls A, Prockop DJ, Myers JC. Osteogenesis imperfecta: cloning of a pro- α 2(I) collagen gene with a frameshift mutation. *J Biol Chem* 1984;259:12941–4. [PubMed: 6092353]
- Pope FM, Nicholls AC, McPheat J, Talmud P, Owen R. Collagen genes and proteins in osteogenesis imperfecta. *J Med Genet* 1985;22:466–78. [PubMed: 3001313]
- Renieri A, Seri M, Myers JC, Pihlajaniemi T, Massella L, Rizzoni G, De Marchi M. De novo mutation in the COL4A5 gene converting glycine 325 to glutamic acid in Alport syndrome. *Hum Mol Genet* 1992a;1:127–9. [PubMed: 1363780]
- Renieri A, Seri M, Myers JC, Pihlajaniemi T, Sessa A, Rizzoni G, De Marchi M. Alport syndrome caused by a 5' deletion within the COL4A5 gene. *Hum Genet* 1992b;89:120–1. [PubMed: 1577459]
- Resendes BL, Robertson NG, Szustakowski JD, Resendes RJ, Weng Z, Morton CC. Gene discovery in the auditory system: characterization of additional cochlear-expressed sequences. *J Assoc Res Otolaryngol* 2002;3:45–53. [PubMed: 12083723]
- Resendes BL, Kuo SF, Robertson NG, Giersch AB, Honrubia D, Ohara O, Adams JC, Morton CC. Isolation from Cochlea of a Novel Human Intronless Gene with Predominant Fetal Expression. *J Assoc Res Otolaryngol* 2004;5:185–202. [PubMed: 15357420]
- Richards AJ, Yates JR, Williams R, Payne SJ, Pope FM, Scott JD, Snead MP. A family with Stickler syndrome type 2 has a mutation in the COL11A1 gene resulting in the substitution of glycine 97 by valine in α 1 (XI) collagen. *Hum Mol Genet* 1996;5:1339–43. [PubMed: 8872475]
- Robertson NG, Khetarpal U, Gutierrez-Espeleta GA, Bieber FR, Morton CC. Isolation of novel and known genes from a human fetal cochlear cDNA library using subtractive hybridization and differential screening. *Genomics* 1994;23:42–50. [PubMed: 7829101]
- Robertson NG, Skvorak AB, Yin Y, Weremowicz S, Johnson KR, Kovatch KA, Battey JF, Bieber FR, Morton CC. Mapping and characterization of a novel cochlear gene in human and in mouse: a positional candidate gene for a deafness disorder, DFNA9. *Genomics* 1997;46:345–354. [PubMed: 9441737]
- Robertson NG, Heller S, Lin JS, Resendes BL, Weremowicz S, Denis CS, Bell AM, Hudspeth AJ, Morton CC. A Novel Conserved Cochlear Gene, OTOR: Identification, Expression Analysis, and Chromosomal Mapping. *Genomics* 2000;66:242–248. [PubMed: 10873378]
- Robertson NG, Lu L, Heller S, Merchant SN, Eavey RD, McKenna M, Nadol JB Jr, Miyamoto RT, Linthicum FH Jr, Lubianca Neto JF, Hudspeth AJ, Seidman CE, Morton CC, Seidman JG. Mutations in a novel cochlear gene cause DFNA9, a human nonsyndromic deafness with vestibular dysfunction. *Nature Genet* 1998;20:299–303. [PubMed: 9806553]
- Sajan SA, Warchol ME, Lovett M. Towards a Systems Biology of Mouse Inner Ear Organogenesis: Gene Expression Pathways, Patterns and Network Analysis. *Genetics*. 2007
- Skvorak AB, Weng Z, Yee AJ, Robertson NG, Morton CC. Human cochlear expressed sequence tags provide insight into cochlear gene expression and identify candidate genes for deafness. *Hum Mol Genet* 1999;8:439–52. [PubMed: 9949203]
- Skvorak AB, Robertson NG, Yin Y, Weremowicz S, Her H, Bieber FR, Beisel KW, Lynch ED, Beier DR, Morton CC. An ancient conserved gene expressed in the human inner ear: identification, expression analysis, and chromosomal mapping of human and mouse antequitin (ATQ1). *Genomics* 1997;46:191–9. [PubMed: 9417906]
- Smeets HJ, Melenhorst JJ, Lemmink HH, Schroder CH, Nelen MR, Zhou J, Hostikka SL, Tryggvason K, Ropers HH, Jansweijer MC, et al. Different mutations in the COL4A5 collagen gene in two patients with different features of Alport syndrome. *Kidney Int* 1992;42:83–8. [PubMed: 1635357]
- Street VA, Kallman JC, Robertson NG, Kuo SF, Morton CC, Phillips JO. A novel DFNA9 mutation in the vWFA2 domain of COCH alters a conserved cysteine residue and intrachain disulfide bond formation resulting in progressive hearing loss and site-specific vestibular and central oculomotor dysfunction. *Am J Med Genet A* 2005;139:86–95. [PubMed: 16261627]
- Svensson L, Aszodi A, Reinholt FP, Fassler R, Heinegard D, Oldberg A. Fibromodulin-null mice have abnormal collagen fibrils, tissue organization, and altered lumican deposition in tendon. *J Biol Chem* 1999;274:9636–47. [PubMed: 10092650]

- Tasheva ES, Koester A, Paulsen AQ, Garrett AS, Boyle DL, Davidson HJ, Song M, Fox N, Conrad GW. Mimecan/osteoglycin-deficient mice have collagen fibril abnormalities. *Mol Vis* 2002;8:407–15. [PubMed: 12432342]
- Toriello, HV.; Reardon, W.; Gorlin, RJ. Hereditary Hearing Loss and Its Syndromes. 2. Oxford University Press; Oxford: 2004.
- Usami S, Takahashi K, Yuge I, Ohtsuka A, Namba A, Abe S, Fransen E, Patthy L, Otting G, Van Camp G. Mutations in the COCH gene are a frequent cause of autosomal dominant progressive cochleo-vestibular dysfunction, but not of Meniere's disease. *Eur J Hum Genet* 2003;11:744–8. [PubMed: 14512963]
- Van Camp G, Snoeckx RL, Hilgert N, van den Ende J, Fukuoka H, Wagatsuma M, Suzuki H, Smets RM, Vanhoenacker F, Declau F, Van de Heyning P, Usami S. A new autosomal recessive form of Stickler syndrome is caused by a mutation in the COL9A1 gene. *Am J Hum Genet* 2006;79:449–57. [PubMed: 16909383]
- Vikkula M, Mariman EC, Lui VC, Zhidkova NI, Tiller GE, Goldring MB, van Beersum SE, de Waal Malefijt MC, van den Hoogen FH, Ropers HH, et al. Autosomal dominant and recessive osteochondrodysplasias associated with the COL11A2 locus. *Cell* 1995;80:431–7. [PubMed: 7859284]

Figure 1.

Osteoglycin is highly expressed in the human fetal cochlea. Autoradiograph of Northern blot of 10 micrograms of total RNAs extracted from human fetal brain, thymus, cochlea, spleen, lung, striated muscle (tongue), testis, liver, kidney, bone marrow, and skeletal muscle hybridized with a human *OGN* probe. High-level expression of the expected ~4 kb transcript of *OGN* is seen in the cochlea. The origin of the apparent ~1.8 kb signal is unknown and may reflect background excluded from the 18S region of the gel or an alternative splice product. 28S and 18S rRNAs are shown in the ethidium bromide-stained gel in the bottom panel for relative assessment of RNA quantity.

Figure 2.

A bar graph depicting the expression levels of the probe sets, 1419662_at (white) and 1419663_at (black), representing the *Ogn* transcript, using the Affymetrix mouse M0E430A_2.0 gene chip. Tissue from inner ear epithelia, cochlea, vestibule, utricle and ampullae, saccule, and outside the inner ear were dissected at the developmental stages noted and *Ogn* expression was determined as described in Sajan et al. (2007). *Ogn* expression increased in all tissues during embryonic development.

Figure 3.

Analysis of cochlear function via distortion product otoacoustic emissions (DPOAEs) and auditory brainstem responses (ABRs) shows no significant effects of *Ogn* deficiency. *Ogn*^{-/-} (white circles) and *Ogn*^{+/+} (black circles) mice in a CBA/CaJ background were tested at 6 months of age. Data are group means ($\pm$ S.E.M.): *Ogn*^{-/-} (n=3), *Ogn*^{+/+} (n=5).

A. Mean DPOAE thresholds: threshold is defined as the primary level required to produce a DPOAE of 0 dB SPL.

B. Mean amplitude-vs. level functions for DPOAEs (*f*₂ = 32 kHz)

C. Mean ABR thresholds: threshold is defined by visual inspection of stacked ABR waveforms at all stimulus levels.

D. Mean amplitude-vs. level functions for ABRs (stimuli at 32 kHz)

Table 1
Genes with highest expression in cochlea vs. universal human reference RNA

Rank	Gene name	Symbol	Accession Number	Fold Change	Chromosomal Locus
1	collagen, type 3, alpha 1	COL3A1	NM_000090	91	2q31
2	prostaglandin D2 synthetase 21KD	PTGDS	NM_000954	40	9q34.2
3	collagen, type 1, alpha 2	COL1A2	NM_000089	32	7q22.1
4	pleiotrophin (heparin binding growth factor 8)	PTN	NM_002825	31	7q33-q34
5	Lin-28 homolog B (C. elegans)	LIN28B	NM_001004317	30	6q21
6	collagen, type 9, alpha 3	COL9A3	NM_001853	20	20q13.3
7	collagen, type 11, alpha 2	COL11A2	NM_080679	17	6p21.3
8	laminin, alpha 2 (merosin)	LAMA2	NM_000426	11	6q22-q23
9	myotubularin related protein 3	MTMR3	NM_153051	11	22q12.2
10	osteoglycin (mimecan)	OGN	NM_014057	10	9q22

Table 2 Genes with expression patterns in the developing mouse utricle similar to that of *Ogn*

Probe Set ID	Gene Symbol	Gene Title
1416121_at	Lox	lysyl oxidase
1416136_at	Mmp2	matrix metalloproteinase 2
1416342_at	Tnc	tenascin C
1416572_at	Mmp14	matrix metalloproteinase 14 (membrane-inserted)
1418049_at	Ltbp3	latent transforming growth factor beta binding protein 3
1418464_at	Matn4	matrilin 4
1418599_at	Col11a1	procollagen, type XI, alpha 1
1418670_s_at	Hspg2	perlecan (heparan sulfate proteoglycan 2)
1419089_at	Timp3	tissue inhibitor of metalloproteinase 3
1419613_at	Col7a1	procollagen, type VII, alpha 1
1419672_at	Spock1	sparc/osteonectin, cwcv and kazal-like domains proteoglycan 1
1423578_at	Col11a2	procollagen, type XI, alpha 2
1423885_at	Lamc1	laminin, gamma 1
1425475_at	Col4a5	procollagen, type IV, alpha 5
1426251_at	Cpz	carboxypeptidase Z
1427168_a_at	Col14a1	procollagen, type XIV, alpha 1
1427986_a_at	Col16a1	procollagen, type XVI, alpha 1
1428571_at	Col9a1	procollagen, type IX, alpha 1
1434667_at	Col8a2	procollagen, type VIII, alpha 2
1435026_at	Spock2	sparc/osteonectin, cwcv and kazal-like domains proteoglycan 2
1435990_at	Adams2	a disintegrin-like and metalloproteinase (reprolysin type) with thrombospondin type 1 motif, 2
1436678_at	Sgcb	sarcoglycan, beta (dystrophin-associated glycoprotein)
1437462_x_at	Mmp15	matrix metalloproteinase 15
1438779_at	Col4a3	procollagen, type IV, alpha 3
1438937_x_at	Ang1	angiogenin, ribonuclease A family, member 1
1438966_x_at	Fmod	Fibromodulin
1442063_at	Adamts1	ADAMTS-like 1
1446326_at	Col1a2	procollagen, type I, alpha 2
1447819_x_at	Col8a1	procollagen, type VIII, alpha 1
1448323_a_at	Bgn	Biglycan
1448392_at	Sparc	secreted acidic cysteine rich glycoprotein
1448421_s_at	Aspn	Asporin
1448469_at	LOC630776///Nid1	nidogen 1///similar to Nidogen precursor (Entactin)
1448755_at	Col15a1	procollagen, type XV
1448870_at	Ltbp1	latent transforming growth factor beta binding protein 1
1449286_at	Ntn1	netrin G1
1449581_at	Emid1	EMI domain containing 1
1449979_a_at	Spock3	sparc/osteonectin, cwcv and kazal-like domains proteoglycan 3
1450673_at	Col9a2	procollagen, type IX, alpha 2
1451241_at	Lamb1-1	laminin B1 subunit 1
1451932_a_at	Adamts4	ADAMTS-like 4
1451978_at	Lox1	lysyl oxidase-like 1
1452106_at	Npnt	Nephronectin
1453418_at	Col24a1	procollagen, type XXIV, alpha 1
1453586_at	Entpd1	ectonucleoside triphosphate diphosphohydrolase 1
1455262_at	Thsd4	thrombospondin, type I, domain containing 4
1455494_at	Col1a1	procollagen, type I, alpha 1
1460734_at	Col9a3	procollagen, type IX, alpha 3