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# Ion Channel Gene Expression in the Inner Ear

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## ABSTRACT

The ion channel genome is still being defined despite numerous publications on the subject. The ion channel transcriptome is even more difficult to assess. Using high-throughput computational tools, we surveyed all available inner ear cDNA libraries to identify genes coding for ion channels. We mapped over 100,000 expressed sequence tags (ESTs) derived from human cochlea, mouse organ of Corti, mouse and zebrafish inner ear, and rat vestibular end organs to *Homo sapiens*, *Mus musculus*, *Danio rerio*, and *Rattus norvegicus* genomes. A survey of EST data alone reveals that at least a third of the ion channel genome is expressed in the inner ear, with highest expression occurring in hair cell-enriched mouse organ of Corti and rat vestibule. Our data and comparisons with other experimental techniques that measure gene expression show that every method has its limitations and does not per se provide a complete coverage of the inner ear ion channelome. In addition, the data show that most genes produce alternative transcripts with the same spectrum across multiple organisms, no ion channel gene variants are unique to the inner ear, and many splice variants have yet to be annotated. Our high-throughput approach offers a qualitative computational and experimental analysis of ion channel genes in inner ear cDNA collections. A lack of data and incomplete gene annotations prevent both rigorous statistical analyses and comparisons of entire ion channelomes derived from different tissues and organisms.

**Keywords:** hearing, ionic currents, ion channels, genome, EST mapping, expressed sequence tags, splice variants, alternative transcripts

## INTRODUCTION

All fundamental physiological processes are based on the ability of cells to receive, process, and transmit signals. The signals can be as elementary as small ion movements that create electron potentials across the cell membrane by diffusing in and out of cells through ion channels. These channels allow selected ions to flow through the lipid membrane as a result of changes in the transmembrane potential, ligand binding, or when mechanical forces, temperature, or acidity reach a certain threshold.

Numerous channel variations exist, based on the ions that pass through them and the gating stimuli to which they respond. However, many share a common structural design and are built from similar protein motifs (Wei et al. 1996; Shrivastava and Bahar 2006; Guda et al. 2007). Several hundred human genes are known to encode ion channel proteins. Many of these genes produce alternative transcripts either due to different-sized untranslated regions (e.g., multiple polyadenylation signals), which result in altered translational efficiency, or due to alternative splicing of coding regions, which produce variant amino acid sequences (Coetzee et al. 1999; Lipscombe

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2005; Beisel et al. 2007). In addition, protein and transcript variants can be the result of multiple transcription start sites and alternative RNA editing sites (Ayoubi and Van De Ven 1996). Protein isoforms can substitute for each other and interact in diverse ways, reassembling into complexes with distinct sensitivities and conductance properties.

Many reviews and research articles have been dedicated to the ion channels of various tissues in health and disease (Ackerman and Clapham 1997; Shieh et al. 2000; Niemeyer et al. 2001; Bradding et al. 2003; Rosenthal and Gilly 2003; Jentsch et al. 2004; Kass 2005). Faulty ion channels are the etiology of numerous channelopathies including blindness, cystic fibrosis, diabetes, cardiac arrhythmias, hypertension, respiratory dysfunction, malignant hyperthermia, neurological and psychiatric diseases, and gastrointestinal disorders (Kass 2005; Ashcroft 2006). Directly or indirectly, ion channels are targets for many drugs in today's market (Suh and Yuspa 2005; Tai 2005) and are valuable in nanotechnology applications (Karnik et al. 2005).

Ion channels play an important role in signal transmission, and their malfunction can lead to both hearing and vestibular dysfunction (Coucke et al. 1999; Holt and Corey 1999; Kharkovets et al. 2000; Hunter 2001; Nicolas et al. 2001; Casimiro et al. 2001; Ashmore 2002; Dumont and Gillespie 2003; Marcotti et al. 2003; Rüttiger et al. 2004; Wangemann et al. 2004). Studies of voltage-gated ion channel expression in inner ear tissues (Adamson et al. 2002; Davis 2003) and expression of ionic currents during the development of hearing (Fuchs and Sokolowski 1990; Mammano and Ashmore 1996; Kros et al. 1998; Michna et al. 2003) provide evidence for the fundamental importance of ion channels in defining cellular specialization.

Here, we present hearing-related ion channel transcripts identified in both human and mouse inner ear cDNA collections and report the existence of novel ion channel gene splice variants. Most of these genes have orthologs in zebrafish and rat. We have conservatively chosen genes mapping to orthologous loci from more than one organism to eliminate potential genomic contaminants. Our findings represent a listing of ion channels produced in the ear in the highest number of copies. All of these genes and their variants are expressed in multiple species and tissues, thus representing components of conserved signal transduction circuitry. We also include a table of all ion channel genes found in any one expressed sequence tag (EST) collection from inner ear of human, mouse, rat, or zebrafish, producing the most comprehensive tabulation of hearing and balance relevant ion channel transcripts possible using these resources.

## METHODS

### cDNA libraries, genome, and ontology resources

To study gene expression profiles of the inner ear, we have mapped the sequences of transcripts from the only two available non-normalized, unsubtracted inner ear derived datasets: 15,048 ESTs from a human fetal cochlear cDNA library (Skvorak et al. 1999; Resendes et al. 2002) and 19,749 sequences derived from a mouse organ of Corti cDNA library (Pompeia et al. 2004). A portion of these sequences is available in GenBank (http://www.ncbi.nlm.nih.gov/Genbank/) and UniGene databases (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=UniGene) as the Morton Fetal Cochlear cDNA Library (dbEST Library ID. 371; 16-22 weeks gestation) and the Mouse Organ of Corti cDNA Library (dbEST Library ID.10920; postnatal days 5–13), respectively. Mouse sequences are available also as an interactive web-based public database at NEIBank (http://neibank.nei.nih.gov).

We analyzed ESTs from publicly available normalized and/or subtracted inner ear libraries: 3,405 ESTs from a normalized fetal human cochlear library (dbEST ID.18222, Hamptonet et al. 2005, unpublished), 22,576 ESTs from a subtracted cDNA library made from 7-week and older postnatal mouse inner ear (RIKEN mouse dbEST library ID.9974; Okazaki et al. 2002; Beisel et al. 2004), 17,468 sequence reads from a subtracted embryonic zebrafish inner ear cDNA library (dbEST Library ID.10504; Coimbra et al. 2002), 1,199 EST sequences from the subtracted mouse organ of Corti library (dbEST Library ID.14415, Gerhard et al. unpublished), 1,873 ESTs from a normalized library of newborn mouse inner ear transcripts (Soares NMIE dataset, dbEST Library ID.4088, Klockars et al. 2003), and 20,090 ESTs from a normalized cDNA library made from rat vestibule (Wackym-Soares dataset, dbEST Library ID.16641, Roche et al. 2005).

Figure 1 illustrates the numbers of ion channel transcripts from the unnormalized human cochlear and mouse organ of Corti datasets. We have compared our findings to ion channel transcript frequencies extracted from the entire UniGene collection, including unnormalized libraries from brain (dbEST 11268, NIH_MGC_144: Strausberg et al. 2002, 10,700 unique clones), liver (dbEST ID 7065, NCI_CGAP_ Li9: Strausberg et al. 2002, 28,448 EST sequences, 28,331 unique clones) and kidney (dbEST 539, Barstead MPLRB1: Marra et al. unpublished; 8,060 sequences, 6,749 unique clones), massively parallel signature sequencing (MPSS, Peters et al. 2007), and rat and mouse microarrays (Rivolta et al. 2002; Lin et al. 2003; Morris et al. 2005).

All sequences were aligned to the NCBI Human Genome build 35.1 (June 2004), Mouse build 34

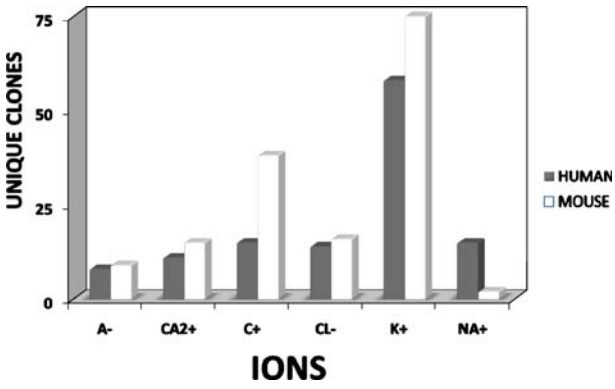


**FIG. 1.** Numbers of unique clones mapped to ion channel genes in non-normalized human (cochlea) and mouse (organ of Corti) cDNA libraries. *A–* Anion porins (nonselective anions), $Ca^{2+}$ calcium channels, *Cl–* chloride channels, $K^+$ potassium channels, $Na^+$ sodium channels, *C+* nonselective cation channels, many of which are mechanosensitive.

(March 2005), Rat build 3.4 (November 2004), and Zebrafish build 1.1 (December 2004); cDNA libraries were from UniGene *Homo sapiens* build #186 and *Mus musculus* build #148 (both of October 2005). Mappings were corrected according to newer genome builds H.s. 36.1 (March 2006), M.m. 36 (February 2006) D.r. Zv6 (March 2006), GenBank Release 153 (May 2006), and UniGene builds 190 (*H. sapiens*), 152 (*M. musculus*), and 90 (*Danio rerio*), all of May 2006. The revised manuscript was adjusted against the NCBI Reference Sequence (RefSeq) release 21 (January 2007), and Genbank release 158 (February 2007), and UniGene builds #200 for *H. sapiens* (January 2007, #161 for *M. musculus* (December 2006), #160 for *Rattus norvegicus* (December 2006), and #100 for *D. rerio* (January 2007).

Transcript variants were compared to those available in RefSeq (http://www.ncbi.nlm.nih.gov/RefSeq/); Ensemble (http://www.ensembl.org/index.html); UniProt: (http://www.pir.uniprot.org/); Vertebrate Genome Annotation Database, Vega (http://vega.sanger.ac.uk/); and ECgene (http://www.genome.ewha.ac.kr/ECgene/) and identified by the programs Twinscan (http://genes.cs.wustl.edu/) and SGP (http://genome.imim.es/genepredictions/index.html), based on mouse–human homology.

We selected all genes annotated with the term "ion channel activity" (GO:0005216) or any of its lower-level terms (see Fig. 2 and Supplementary Material) from the Gene Ontology (GO) database (http://www.geneontology.org/) and resources providing GO annotations (GO Annotation @ EBI, GOA (http://www.ebi.ac.uk/GOA/); Entrez Gene, NCBI (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene); Mouse Genome Informatics Resource, MGI (http://www.informatics.jax.org/); Rat Genome Database, RGD (http://rgd.mcw.edu/), and the Zebrafish Information Network, ZFIN (http://zfin.org), as of December 2006. Note that these are evolving databases and some annotations will change over time.

## Computational analysis of EST data

To map EST sequences directly to the corresponding genomes, we downloaded and installed BLAT, the BLAST-like alignment tool (Kent 2002). Genomic contaminant transcripts were determined by the presence of a poly(A) tail in an EST, and the lack of a poly(A) signal, or by the presence of a poly(A)/poly(T) stretch in the genomic sequence flanking the mapped EST sequence (Gabashvili et al. 2005).

To determine significant differences in EST frequencies across multiple libraries, the data, which consisted of unique clone counts for ion channel genes and all genes in cDNA libraries, were arranged as 2×2 contingency tables for applying a Fisher's exact test (Supplementary Table S2). The general Chi-square test was used to compare the ratio of representative ESTs for each gene in each library. Perl and Matlab scripts were written to automate various data download and analysis tasks.

## RT-PCR of mouse tissue

RNA was prepared from dissected mouse tissues (brain, cochlea, kidney and liver) using a Qiagen (Valencia, CA) RNeasy kit according to the manufacturer's instructions. Universal Reference RNA (Stratagene, La Jolla, CA) consisted of total RNA derived from 11 different mouse cell lines, including B-lymphocyte, T-lymphocyte, macrophage, kidney, liver, mammary gland, muscle, skin, testis and two embryonic lines. Equal amounts of total RNA were reverse transcribed using the Superscript III kit (Invitrogen, Carlsbad, CA) with oligo-dT as primer following the manufacturer's protocols. To control for genomic contamination, parallel reactions were performed without reverse transcriptase. One µl of each reaction (+/- RT) was used in PCR amplification reactions with primers specific to the known 3′ UTR of *Trpm3 (BQ563941, Mm.*422907*)*, the novel 3′ UTR of *Trpm3* identified in this study, the known 3′ UTR of *Kcna2 (*CF744586, *Mm.56930)*, the novel 3′ UTR of *Kcna2*, the known 3′ UTR of the longer *Kcnma1* variant (AK135652, *Mm.*343607) and 3′ of the shorter mouse *Kcnma1* variant identified in this work *(BQ570163, Mm.*343607*)*. We note that a similar splice variant of this gene is annotated in human and rat genomes, but not in mouse. Primer sequences for each template consisted of (5′ to 3′): *Trpm3*, known: forward - AGGGCTAAATT GCAAAATGAAA, reverse - TGACCAATTACAGAGGTG TTGG; *Trpm3*, novel: forward - TACCAGTGTGGCATT GATTCTC, reverse - GCACCTATGAAGGGGATGAT

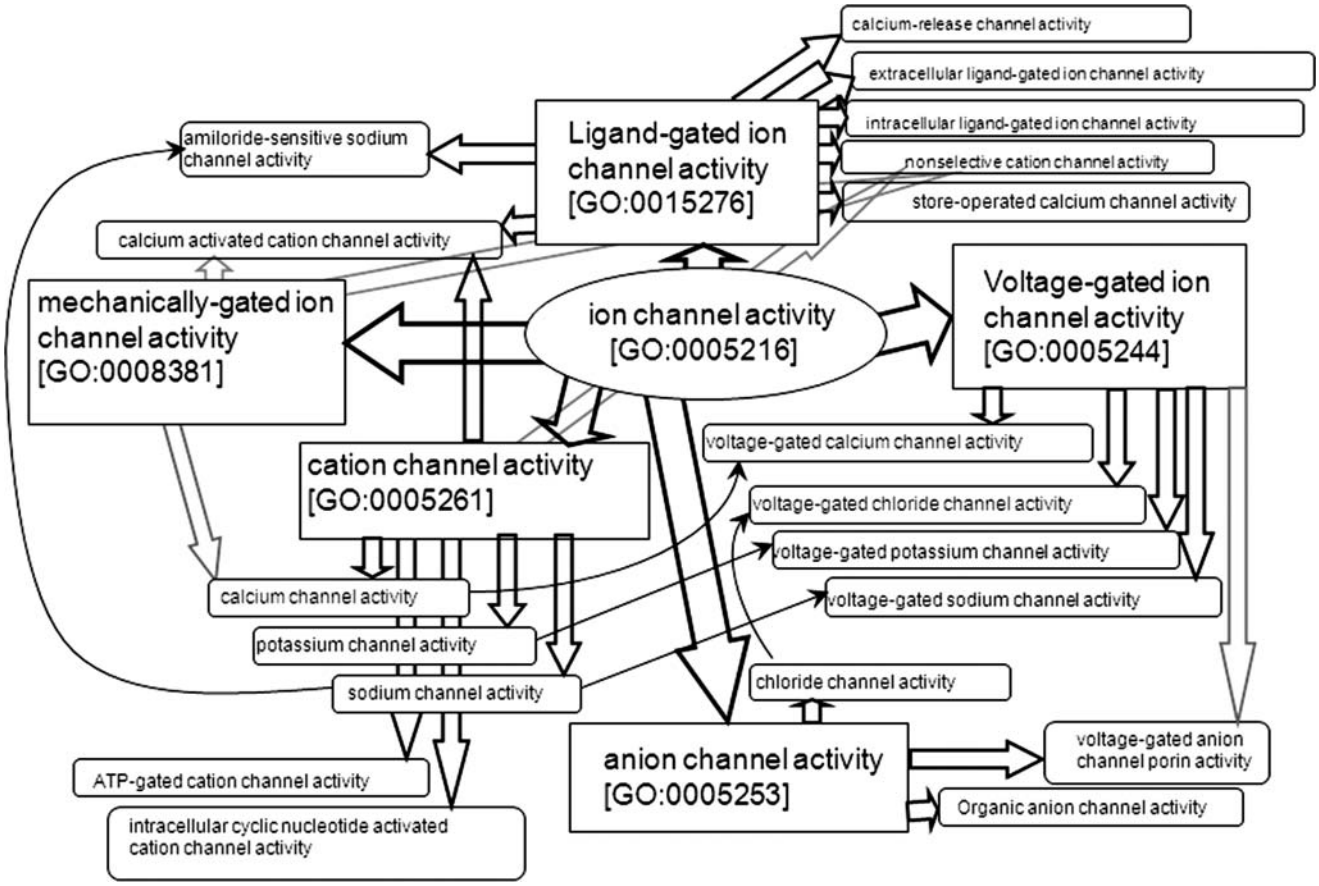


**FIG. 2.** Gene ontology relationships among concepts mapped to the term "ion channel activity." *Grey links* show relationships known from the ion channel literature that were added to the analysis in this study. Note that the concept "channel regulator activity" is not included, as it belongs to "auxiliary transport protein activity" and not to "channel or pore class transporter activity," a parent term of "ion channel activity".

TA; *Kcna2*, known: forward - GTCATTCTCGGTTTTGT GTTGA, reverse - CTTGGCAATTCATATTTGGTCA; *KCNA2*, novel: forward - TAGTTCCTGGTCACCCATAT CC, reverse - ATGGGTGTGGACATTTTATGGT; *Kcnma1 variant 1*,: forward - GTCTGTCTCATTCCTCC CACTC, reverse - CATTTTGATTTTGCATTGCTGT; Kcnma1 variant 2,: forward - AAGAGCTCCTCCGTC CACTC, reverse - TTTTCATTGCTTTTTGGTTGCT; Otor: forward - TTAGCTTTCAGCGAGGAGAATC, reverse - CATTCTTCTATCCAAGGGCATC; *Gapdh*: forward - GGGTGGAGCCAAACGGGT, reverse - GGAGTT GCTGTTGAAGTCGCA. Amplicons for the known vs novel transcripts of each gene were designed to be of near identical size so amplification kinetics would be comparable. Reactions were performed with increasing numbers of cycles of amplification until expression level differences were observed between different tissues. Equal amounts (14 μl) of each reaction were loaded onto agarose gels. PCR reactions of 31 cycles were performed for *Trpm3*, *Kcna2* and *Kcnma1*. PCR reactions of 28 cycles were performed for both *Gapdh* (glyceraldehyde-3-phosphate dehydrogenase), a positive control for each tissue, and *Otor*, to demonstrate cochlear-specific transcript amplification. Each PCR product was verified by sequencing. Sequence variants were deposited in GenBank.

## RESULTS

### Ion channel genome

The function of most genes annotated as ion channels in the Gene Ontology database (genes annotated with the term "ion channel activity" or its child term, see Fig. 2 and Methods) was inferred computationally and not experimentally (such as direct assays, expression, interaction, or mutation studies). Supplementary Table 1 lists all genes annotated with ion channel activity in at least two vertebrate organisms, of which there are 331 entries with 313 annotated in the human genome. We assume that there is experimental evidence supporting ion channel annotation if either "Function Evidence" (ion channel activity) or "Component Evidence" (ion channel complex) is annotated with one of the experimental GO Evidence Codes in at least one of the organisms listed. Even with such assumptions, there are more computationally anno-

tated ion channel orthologs than experimentally annotated ones (172 vs 159 among vertebrates). We note, however, that not all of these annotations are correct; experimental evidence may exist, but not documented in several cases. This table was compiled in December 2006. Over a three-month period, the number of annotations citing experimental evidence noticeably increased. This is seen from Table 1 (compiled in March 2007), which lists ion channel genes expressed in the inner ear, 162 of which are annotated experimentally vs 111 computational annotations. Over a six-month period, the number of genes annotated as ion channels changed from 342, 329, and 116 to 356, 350, and 303 in human, mouse, and zebrafish, respectively. In addition, there were 274 ion channel genes annotated in *Bos taurus*, 187 in *Gallus gallus*, and 289 in *R. norvegicus*. Many more vertebrate ion channel genes are available in the Entrez Gene database, but they are not annotated with GO terms (e.g. *Pan troglodytes*, *Sus scrofa*, or *Taeniopygia guttata* genomes). The number of genes linked to "ion channel activity" and shared among genomes increased from 229 to 261 for genes common to human and mouse, from 49 to 52 for genes common to human, mouse and zebrafish and from 20 to 32 orthologs in four species: human, mouse, rat and zebrafish. We note that this list will continue to evolve; its current incompleteness is due to lags in matching protein accessions to Gene IDs, incomplete Gene Ontology hierarchy and the lack of functional annotations.

## Ion channel transcripts in tissue-specific EST libraries

**Human.** Our EST analysis and gene mapping results reveal that ion channels represent only a small fraction of genes expressed in cells. Even in the most active signal-conducting tissues such as brain, only about 2% of expressed genes are ion channels. Out of 15,048 ESTs from the human cochlea (10,647 clones), 169 ESTs (121 clones) align to 42 ion channel genes. We consider 28 of the 169 ESTs (20 clones) to be genomic contaminants. We excluded seven more human ESTs from further consideration as their orientation did not match the direction of the gene to which they map (all these ESTs are marked as "suboptimal member of this cluster" in UniGene). In the end, 134 human cochlear ESTs were derived reliably from ion channel transcripts.

**Mouse.** Another non-normalized dataset examined in the present study, a mouse organ of Corti library, contains 19,749 sequences, mainly 5′ ESTs, corresponding to 19,075 unique clones. We have identified 157 ion channel ESTs (61 ion channel genes) in this dataset, each of which is from a unique clone. We did not detect genomic contaminations in this dataset, primarily because of the 5′ nature of all the sequences. Among the ion channel genes identified, 12 (8 clones) in human and 9 ESTs in mouse represent non-selective channels conducting anions, 14 (11 clones) and 15 ESTs map to $Ca^{2+}$ channels, 22 (15) and 38 ESTs map to non-selective channels conducting cations, 17 (14) and 16 ESTs map to $Cl^-$ channels, 84 (58) and 77 ESTs map to $K^+$ channels, and 20 (15) and 2 ESTs represent $Na^+$-conducting channels, in human and mouse respectively. Normalized human cochlear dataset (ID.18222) ESTs map to only 4 ion channel genes, but two of them (*KCTD6* and *GRIK4*) are not present in the unnormalized library.

**Rat and zebrafish.** Over three hundred transcripts (5′ ends of 225 unique clones) of the normalized rat vestibular cDNA library map to 115 genes annotated as ion channels in *Rattus norvegicus*. This number constitutes about 38% of all genes annotated as ion channels in the rat genome and about 30% of the putative vertebrate ion channel genome.

The zebrafish dataset contains 175 ion channel or ion channel-like ESTs (121 unique clones) mapped to 36 genes. Less than half of the human and mouse ESTs map to genes identified in both datasets. Even fewer human transcripts map to ion channel genes from the zebrafish inner ear library, rat vestibular cDNA library, or genes detected in cochlear mouse microarrays. Only two ion channel genes, *KCNMA*1 and *CLIC4*, are found in the cDNA libraries from all four organisms and are properly annotated in all these organisms.

Table 1 lists all ion channel genes found in any one of the studied libraries and presents several additional genes detected in microarray, MPSS, and reverse transcriptase-polymerase chain reaction (RT-PCR) experiments (as of March 2007). It includes possible genomic contaminations and suboptimal alignments and presents even the weakest candidates. Obviously, it is not possible to directly compare cochlea, organ of Corti, entire inner ear, and vestibular systems to each other. Contributing to the variation between datasets is the limitation that different libraries are constructed using various tissue isolation, RNA extraction, and amplification techniques. Moreover, large-scale experiments are expected to miss genes expressed in low numbers. We find that any one of the high-throughput or low-throughput techniques applied to ear tissues identifies expression of genes not detected by alternative methods. Nonetheless, comparison of overlapping ion channels shed light on the most highly expressed ion channel genes of the inner ear. Ion channel transcripts identified in the only two unnormalized datasets of human and mouse are listed in Table 2

**TABLE 1**

Ion channel genes expressed in the inner ear as of March 2007

| *Expressed* | *Genome* | *Gene symbol* | *Full name* | *GOE* | *GO annotation* |
|---|---|---|---|---|---|
| R | DHM | *ABCC4* | ATP-binding cassette, sub-family C (CFTR/MRP), member 4 | E | $Cl^-$ channel activity |
| HMR | R | *ABCC9* | ATP-binding cassette, subfamily C (CFTR/MRP), member 9 | E | $K^+$ channel activity, ATP-sensitive $K^+$ channel complex |
| *Microarray* | R | *ABP1* | Amiloride binding protein 1 (amine oxidase, $Cu^{2+}$-containing) | C | Amiloride-sensitive $Na^+$ channel activity |
| MR | DHMR | *ACCN1* | Amiloride-sensitive cation channel 1, neuronal (degenerin) | E | Amiloride-sensitive $Na^+$ channel activity, $Na_v$ channel/inhibitor activity, ICA |
| DMR | DGHMR | *ACCN2* | Amiloride-sensitive cation channel 2, neuronal | E | Amiloride-sensitive $Na^+$ channel activity, ICA |
| DM | HMR | *ACCN3* | Amiloride-sensitive cation channel 3 | E | Amiloride-sensitive $Na^+$ channel activity, ICA |
| *Microarray* | MR | *AI118078* | Expressed sequence AI118078 | C | ICA |
| HR | HR | *ANXA7* | Annexin A7 | C | $Ca_v$ channel activity |
| R | M | *AVEN* | Apoptosis, caspase activation inhibitor | C | $Ca^{2+}$ channel activity, ICA |
| M | HMR | *BSND* | Bartter syndrome, infantile, with sensorineural deafness (Barttin) | E | $Cl^-$ channel activity |
| R | HM | *BTBD10* | BTB (POZ) domain containing 10 | C | $K_v$ channel activity/complex |
| R | BDHMR | *CACNA1A* | $Ca_v$ channel, P/Q type, α1A | E | ICA, $Ca_v$ channel activity/complex |
| *Microarray* | BGHM | *CACNA1B* | $Ca_v$ channel, N type, α1B | E | ICA, $Ca_v$ channel activity/complex |
| MR | BDGHMR | *CACNA1C* | $Ca_v$, L type, α1C | E | $Ca^{2+}$ channel activity, $Ca^{2+}$ channel inhibitor activity, $Ca_v$ channel activity/complex, |
| *Microarray* | BDHM | *CACNA1D* | $Ca_v$, L type, α1D | E | Dihydropyridine-sensitive $Ca^{2+}$ channel activity, $Ca_v$ channel activity/complex |
| M | BHMR | *CACNA1E* | $Ca_v$, R type, α1E | E | ICA, $K_v$ channel activity, $Ca_v$ channel activity/complex |
| HM | BHMR | *CACNA1G* | $Ca_v$, T type, α1G | E | ICA, $Ca_v$ channel activity/complex |
| HMR | HMR | *CACNA2D1* | $Ca_v$, α2/δ1 | E | $Ca^{2+}$ channel activity, $Ca_v$ channel activity/complex |
| R | R | *CACNA2D3* | $Ca_v$, α2/δ3 | E | $Ca^{2+}$ channel regulator activity, $Ca_v$ channel activity/complex |
| R | BDHMR | *CACNB1* | $Ca_v$, β1 | E | $Ca^{2+}$ channel activity, $Ca^{2+}$ channel inhibitor activity, dihydropyridine-sensitive $Ca_v$ channel activity |
| HMR | BDHMR | *CACNB2* | $Ca_v$, β2 | E | Amiloride-sensitive $Na^+$ channel activity, $Ca_v$ channel activity |
| MR | BHMR | *CACNB3* | $Ca_v$, β3 | E | $Ca_v$ channel activity |
| HM | BGHMR | *CACNB4* | $Ca_v$, β4 | E | IBID |
| *MPSS* | GHMR | *CACNG4* | $Ca_v$, γ4 | E | Voltage-gated channel complex, $Ca_v$ activity/complex |
| R | D | *CALB2* | Calbindin 2, (calretinin) | C | $Ca^{2+}$ channel activity, $Ca^{2+}$ channel inhibitor activity |
| HMR | D | *CALM1* | Calmodulin 1 | C | $Ca^{2+}$ channel activity, $Ca^{2+}$ channel regulator activity, channel-conductance-controlling ATPase activity |
| DHMR | D | *CALM2* | Calmodulin 2 | C | $Ca^{2+}$ channel activity, $Ca^{2+}$ channel inhibitor activity |
| DHR | D | *CALM3* | Calmodulin 3 | C | IBID |
| DMR | D | *CALR* | Calreticulin | C | IBID |

**TABLE 1 (continued)**

Ion channel genes expressed in the inner ear as of March 2007

| *Expressed* | *Genome* | *Gene symbol* | *Full name* | *GOE* | *GO annotation* |
|---|---|---|---|---|---|
| *Microarray* | D | *CASQ2* | Calsequestrin 2 | C | IBID |
| HM | D | *CDH1* | Cadherin 1, epithelial | C | IBID |
| *Microarray* | | *CDH10* | Cadherin 10, type 2 (T2-cadherin) | C | IBID |
| HR | D | *CDH11* | Cadherin 11, osteoblast | C | IBID |
| M | D | *CDH15* | Cadherin 15, M-cadherin (myotubule) | C | IBID |
| R | D | *CDH17* | Cadherin 17, LI cadherin (liver-intestine) | C | IBID |
| DR | D | *CDH2* | Cadherin 2, neuronal | C | IBID |
| R | D | *CDH23* | Cadherin-like 23 | C | IBID |
| HR | D | *CDH5* | Cadherin 5 | C | IBID |
| D | D | *CDH6* | Cadherin 6 | C | IBID |
| *Microarray* | BHMR | *CFTR* | Cystic fibrosis transmembrane conductance regulator (ATP-binding cassette subfamily C, member 7) | E | ATP-binding and phosphorylation-dependent Cl– channel activity, channel-conductance-controlling ATPase activity |
| *Microarray* | BDGHMR | *CHRNA1* | Cholinergic receptor, nicotinic, α1 (muscle) | E | ICA, nicotinic ACh-activated cation-selective channel activity/complex |
| H | H | *CHRNA10* | Cholinergic receptor, nicotinic, α10 | E | ICA, nicotinic ACh-activated/gated cation-selective channel activity |
| M | GHMR | *CHRNA4* | Cholinergic receptor, nicotinic, α4 | E | ICA, nicotinic ACh-activated/gated cation-selective channel activity/complex |
| DMR | BDGHMR | *CHRNA7* | Cholinergic receptor, nicotinic, α7 | E | ICA, extracellular ligand-gated ICA, nicotinic ACh-activated cation-selective channel activity |
| M | GHMR | *CHRNA9* | Cholinergic receptor, nicotinic, α9 | E | ICA, extracellular ligand-gated ICA, nicotinic ACh-activated cation-selective channel activity |
| M | BGHMR | *CHRNB4* | Cholinergic receptor, nicotinic, β4 | E | ICA, extracellular ligand-gated ICA, nicotinic ACh-activated cation-selective channel activity |
| *Microarray* | BDHM | *CHRNE* | Cholinergic receptor, nicotinic, ε | E | IBID |
| *Microarray* | BGHMR | *CHRNG* | Cholinergic receptor, nicotinic, γ | E | Nicotinic ACh-activated cation-selective channel activity, nicotinic ACh-gated receptor-channel complex |
| R | D | *CIB2* | $Ca^{2+}$ and integrin binding family member 2 | C | $Ca^{2+}$ channel activity, $Ca^{2+}$ channel inhibitor activity |
| *Microarray* | BM | *CLCA3* | Cl– channel $Ca^{2+}$-activated 3 | C | Cl– channel activity, voltage-gated ICA |
| M | M | *CLCA5* | Cl– channel $Ca^{2+}$-activated 5 | E | Cl– channel activity, ligand-gated ICA |
| M | HMR | *CLCC1* | Cl– channel CLIC-like 1 | E | Cl– channel activity |
| MR | BHMR | *CLCN2* | Cl– channel 2 | E | $Cl_v$– channel activity |
| HR | HMR | *CLCN3* | Cl– channel 3 | E | IBID |
| MR | R | *CLCN4-2* | Cl– channel 4-2 3 | C | $Cl_v$– channel activity, voltage-gated ICA |
| H | BHMR | *CLCN5* | Cl– channel 5 (nephrolithiasis 2, X-linked, Dent disease) | E | $Cl_v$– channel activity |
| M | HM | *CLCN6* | Cl– channel 6 | C | IBID |
| M | BGHM | *CLCN7* | Cl– channel 7 | E | IBID |
| M | HMR | *CLCNKA* | Cl– channel Ka | E | IBID |

**TABLE 1 (continued)**

Ion channel genes expressed in the inner ear as of March 2007

| *Expressed* | *Genome* | *Gene symbol* | *Full name* | *GOE* | *GO annotation* |
|---|---|---|---|---|---|
| MR | BDHMR | *CLIC1* | Cl– intracellular channel 1 | E | Cl– channel activity, $Cl_v$– channel activity |
| R | H | *CLIC2* | Cl– intracellular channel 2 | C | Cl– channel activity, $Cl_v$– channel activity |
| DHMR | BDHMR | *CLIC4* | Cl intracellular channel 4 | E | Cl– channel activity |
| H | BDHMR | *CLIC5* | Cl intracellular channel 5 | E | Cl– channel activity, $Cl_v$– channel activity |
| *Microarray* | DHMR | *CNGA2* | CNG channel α2 | E | ICA |
| D | DR | *CSEN* | Potassium channel–interacting protein KchIP | E | $Ca^{2+}$ channel activity, $Ca^{2+}$ channel inhibitor activity |
| HM | HR | *CUL5* | Cullin 5 | E | $Ca^{2+}$ channel activity |
| M | BHM | *CYBB* | Cytochrome b-245, beta polypeptide (chronic granulomatous disease) | C | $Ca^{2+}$ channel regulator activity. voltage-gated ICA |
| D | D | *DLB* | δB | C | $Ca^{2+}$ channel activity, $Ca^{2+}$ channel inhibitor activity |
| MR | D | *DLD* | δD | E | IBID |
| R | D | *EEF2K* | Elongation factor-2 kinase | C | $Ca^{2+}$ channel activity, $Ca^{2+}$ channel inhibitor activity |
| M | M | *EG622320* | Predicted gene, EG622320 | C | ICA, $K_v$ channel activity, $K_v$ channel complex |
| H | D | *FAT* | FAT tumor suppressor homolog 1 | C | $Ca^{2+}$ channel activity, $Ca^{2+}$ channel inhibitor activity |
| HMR | D | *FBLN1* | Fibulin 1 | C | IBID |
| HM | R | *FGF2* | Fibroblast growth factor 2 | E | $Ca_v$ channel activity |
| R | D | *FKBP7* | FK506 binding protein 7 | C | $Ca^{2+}$ channel activity, $Ca^{2+}$ channel inhibitor activity |
| DHMR | D | *FKBP9* | FK506 binding protein 9 | C | IBID |
| HR | D | *FSTL5* | Follistatin-like 5 | C | IBID |
| MR | HMR | *FXYD1* | FXYD domain containing ion transport regulator 1 (phospholemman) | E | Cl– channel activity, ICA |
| R | BHMR | *FXYD2* | FXYD domain-containing ion transport regulator 2 | E | ICA |
| R | BHM | *FXYD3* | FXYD domain containing ion transport regulator 3 | E | ICA |
| MR | HM | *FXYD5* | FXYD domain-containing ion transport regulator 5 | C | ICA |
| DHMR | BHM | *FXYD6* | FXYD domain containing ion transport regulator 6 | C | ICA |
| R | BGHMR | *GABRA1* | γ-aminobutyric acid (GABA-A) receptor, α1 | E | Extra-cellular ligand-gated ICA |
| M | BHM | *GABRA2* | GABA-A, α2 | C | IBID |
| *Microarray* | BHM | *GABRA3* | GABA-A, α3 | C | IBID |
| *Microarray* | GHMR | *GABRA6* | GABA-A, α6 | E | IBID |
| *Microarray* | BGHMR | *GABRB1* | GABA-A, β1 | E | IBID |
| *Microarray* | HMR | *GABRB2* | GABA-A, β2 | E | IBID |
| *Microarray* | GHM | *GABRB3* | GABA-A, β3 | C | IBID |
| *Microarray* | HM | *GABRD* | GABA-A, δ | C | IBID |
| *Microarray* | BGHMR | *GABRG2* | GABA-A, γ2 | E | IBID |
| *Microarray* | HMR | *GABRG3* | GABA-A γ3 | E | IBID |
| *Microarray* | HM | *GABRQ* | GABA-A, θ | E | IBID |
| *Microarray* | GHM | *GABRR1* | GABA, ρ1 | C | IBID |
| *Microarray* | BGHMR | *GABRR2* | GABA, ρ2 | E | IBID |
| DMR | BDHMR | *GLRA3* | Glycine receptor, α3 | E | ICA, glycin-gated Cl– channel complex |
| MR | BDHMR | *GLRB* | Glycine receptor, β | E | Glycine-gated ICA, glycine-inhibited Cl– channel activity |

**TABLE 1 (continued)**

Ion channel genes expressed in the inner ear as of March 2007

| *Expressed* | *Genome* | *Gene symbol* | *Full name* | *GOE* | *GO annotation* |
|---|---|---|---|---|---|
| H | D | *GPR98* | G protein-coupled receptor 98 | C | ICA, $Ca^{2+}$ channel activity, $Ca^{2+}$ channel inhibitor activity |
| MR | DGHMR | *GRIA2* | Glutamate receptor, ionotropic, AMPA2 | E | ICA, glutamate-gated ICA, $K^+$ channel activity |
| DM | DGMH | *GRIA3* | AMPA3 | E | Glutamate-gated ICA, ICA |
| HMR | BDGHM | *GRIA4* | AMPA4 | C | IBID |
| R | DHM | *GRID2* | Glutamate receptor, ionotropic, δ2 | E | IBID |
| HR | HMR | *GRIK1* | Glutamate receptor, ionotropic, kainate 1 | E | IBID |
| H | HMR | *GRIK2* | Glutamate receptor, ionotropic, kainate 2 | E | IBID |
| MR | GHMR | *GRIN1* | Glutamate receptor, ionotropic, NMDA1 | E | ICA, $K^+$ channel activity, glutamate-gated ICA |
| *Microarray* | | *GRIN2A* | Glutamate receptor, ionotropic, NMDA2A | E | ICA, glutamate-gated ICA, |
| *Microarray* | HMR | *GRIN2C* | Glutamate receptor, ionotropic, NMDA2C | E | IBID |
| M | HMR | *GRIN2D* | Glutamate receptor, ionotropic, NMDA2D | E | IBID |
| R | R | *GRINA* | Glutamate receptor, ionotropic, NMDA-associated | C | Cation channel activity |
| *Microarray* | D | *GUCA1A* | Guanylate cyclase activator 1A | C | $Ca^{2+}$ channel activity, $Ca^{2+}$ channel inhibitor activity |
| MR | DM | *HCN1* | Hyperpolarization-activated CNG $K^+$ channel | C | $Na^+$ channel activity, $K_v$ channel activity |
| M | GHM | *HVCN1* | Hydrogen voltage-gated channel 1 | E | ICA, voltage-gated proton channel activity |
| D | D | *ICN* | Ictacalcin | C | $Ca^{2+}$ channel activity, $Ca^{2+}$ channel inhibitor activity |
| HM | BGHN | *ITPR1* | Inositol 1,4,5-triphosphate receptor, type 1 | E | $Ca^{2+}$ channel activity |
| MR | BHMR | *ITPR2* | Inositol 1,4,5-triphosphate receptor, type 2 | E | $Ca^{2+}$ channel activity, inositol 1,4,5-triphosphate-sensitive $Ca^{2+}$-release channel activity, ICA |
| *Microarray* | BHM | *ITPR3* | Inositol 1,4,5-triphosphate receptor 3 | E | IBID |
| HM | D | *ITSN1* | Intersectin 1 (SH3 domain protein) | C | $Ca^{2+}$ channel activity, $Ca^{2+}$ channel inhibitor activity |
| DMR | HM | *KCMF1* | $K^+$ channel modulatory factor 1 | C | ICA |
| MR | GHMR | *KCNA1* | $K_v$, Shaker-related member 1 | E | $K_v$ channel activity/complex |
| HM | GHMR | *KCNA2* | $K_v$, Shaker-related member 2 | E | IBID |
| *Microarray* | GHMR | *KCNA3* | $K_v$, Shaker-related member 3 | E | IBID |
| *Microarray* | BGHMR | *KCNA4* | $K_v$ channel, Shaker-related member 4 | E | IBID |
| *Microarray* | HMR | *KCNA6* | $K_v$ channel, Shaker-related member 6 | E | IBID |
| MR | GHMR | *KCNA10* | $K_v$ channel, Shaker-related member 10 | E | IBID |
| MR | BGHMR | *KCNAB1* | $K_v$ channel, Shaker-related β1 | E | IBID |
| M | BGHMR | *KCNAB2* | $K_v$ channel, Shaker-related β2 | E | IBID |
| *Microarray* | HMR | *KCNAB3* | $K_v$ channel, Shaker-related β3 | E | IBID |
| M | HMR | *KCNB1* | $K_v$ channel, Shab-related member 1 | E | IBID |
| M | BHMR | *KCNB2* | $K_v$ channel, Shab-related member 2 | E | IBID |
| R | BGHMR | *KCNC1* | $K_v$ channel, Shaw-related member 1 | E | IBID |

**TABLE 1 (continued)**

Ion channel genes expressed in the inner ear as of March 2007

| *Expressed* | *Genome* | *Gene symbol* | *Full name* | *GOE* | *GO annotation* |
|---|---|---|---|---|---|
| R | HMR | *KCNC2* | $K_v$ channel, Shaw-related member 2 | C | IBID |
| R | HMR | *KCNC3* | $K_v$ channel, Shaw-related member 3 | E | IBID |
| M | HM | *KCNC4* | $K_v$ channel, Shaw-related member 4 | E | IBID |
| *Microarray* | GHMR | *KCND2* | $K_v$ channel, Shal-related member 2 | E | IBID |
| R | BHMR | *KCNE1* | $K_v$ channel, Isk-related family, member 1 | E | IBID |
| H | MHR | *KCNG1* | $K_v$ channel, subfamily G, member 1 | C | IBID |
| R | BHMR | *KCNH1* | $K_v$ channel, subfamily H (eag-related), member 1 | E | IBID |
| M | DHMR | *KCNH2* | $K_v$ channel, subfamily H (eag-related), member 2 | E | IBID |
| HM | HMR | *KCNJ10* | $K_{ir}$, subfamily J, member 10 | E | ATP-activated $K_{ir}$ channel activity |
| *Microarray* | DHMR | *KCNJ11* | $K_{ir}$, subfamily J, member 11 | E | IBID |
| *Microarray* | DHMR | *KCNJ12* | $K_{ir}$, subfamily J, member 12 | E | IBID |
| HMR | HMR | *KCNJ16* | $K_{ir}$, subfamily J, member 16 | C | IBID |
| HM | BGHM | *KCNJ2* | $K_{ir}$ channel, subfamily J, member 2 | E | IBID |
| R | GHMR | *KCNJ3* | $K_{ir}$ channel, subfamily J, member 3 | E | G-protein-activated $K_{ir}$ activity, voltage-gated ICA |
| *Microarray* | GHMR | *KCNJ4* | $K_{ir}$ channel, subfamily J, member 4 | E | $K_{ir}$ activity, Kv channel complex |
| *Microarray* | GHMR | *KCNJ5* | $K_{ir}$ channel, subfamily J, member 5 | E | G-protein-activated $K_{ir}$ activity |
| *Microarray* | HMR | *KCNJ6* | $K_{ir}$ channel, subfamily J, member 6 | E | G-protein-activated $K_{ir}$ activity, $K_v$ channel complex |
| M | BDGHMR | *KCNJ8* | $K_{ir}$ channel, subfamily J, member 8 | E | ATP-activated $K_{ir}$ activity |
| *Microarray* | HMR | *KCNJ9* | $K_{ir}$ channel, subfamily J, member 9 | C | G-protein activated $K_{ir}$ activity |
| HMR | GHMR | *KCNK1* | $K^+$ channel, subfamily K, member 1 | E | $K^+$ channel activity |
| H | BH | *KCNK17* | $K^+$ channel, subfamily K, member 17 | E | $K^+$ channel activity, voltage-gated ICA |
| MR | BHMR | *KCNK2* | $K^+$ channel, subfamily K, member 2 | E | ICA, $K^+$ channel activity |
| M | DHM | *KCNK5* | $K^+$ channel, subfamily K, member 5 | E | $K^+$ channel activity |
| D | HMR | *KCNK6 (TWK-6)* | $K_{ir}$ channel, subfamily K, member 6 | C | $K_{ir}$ activity, $K^+$ channel activity, voltage-gated ICA, $K_v$ channel complex |
| DHMR | BGHMR | *KCNMA1* | BK channel, subfamily M, α1 | E | ICA, $K_{Ca}$ activity, $K_v$ channel activity |
| *Microarray* | | *KCNMB1* | BK, subfamily M, β1 | E | ICA, $K_{Ca}$ channel activity |
| H | HMR | *KCNMB2* | BK, subfamily M, β2 | E | $K^+$ channel activity |
| *RT-PCR* | BHMR | *KCNN1* | IK/SK channel, subfamily N, member 1 | E | SK channel activity, $K_v$ channel complex |
| *RT-PCR* | BGHMR | *KCNN2* | IK/SK channel, member 2 | E | SK channel activity |
| R | HMR | *KCNN4* | IK/SK channel, member 4 | E | IK/SK channel activity |
| HM | HMR | *KCNQ1* | $K_v$ channel, KQT-like subfamily, member 1 | E | $K_v$ channel activity, $K_v$ channel complex |
| *RT-PCR* | HMR | *KCNQ2* | $K_v$ channel, subfamily Q, member 2 | E | IBID |
| *RT-PCR* | BHMR | *KCNQ3* | $K_v$ channel, subfamily Q, member 3 | E | IBID |
| M | GHMR | *KCNQ4* | $K_v$ channel, subfamily Q, member 4 | E | IBID |

**TABLE 1 (continued)**

Ion channel genes expressed in the inner ear as of March 2007

| *Expressed* | *Genome* | *Gene symbol* | *Full name* | *GOE* | *GO annotation* |
|---|---|---|---|---|---|
| DM | HMR | *KCNQ5* | $K_v$ channel, subfamily Q, member 5 | E | IBID |
| R | HMR | *KCNS1* | $K_v$ channel, delayed rectifier, subfamily S, member 1 | E | $K^+$ channel regulator activity, $K_v$ channel complex |
| *Microarray* | HM | *KCNS2* | $K_v$ channel, delayed-rectifier, subfamily S, member 2 | C | IBID |
| MR | GHMR | *KCNT1* | $K^+$ channel, subfamily T, member 1 | E | $K_{Ca}$ and $K^+$ channel activity, voltage-gated ICA |
| R | BGHM | *KCTD1* | $K^+$ channel tetramerization domain containing 1 | C | $K_v$ channel activity/complex |
| HR | BDHMR | *KCTD10* | $K^+$ channel tetramerization domain containing 10 | C | IBID |
| M | BHM | *KCTD11* | $K^+$ channel tetramerization domain containing 11 | C | IBID |
| HMR | DHM | *KCTD12* | $K^+$ channel tetramerization domain containing 12 | C | IBID |
| H | HMR | *KCTD13* | $K^+$ channel tetramerization domain containing 13 | C | IBID |
| R | GHM | *KCTD14* | $K^+$ channel tetramerization domain containing 14 | C | IBID |
| MR | BHM | *KCTD15* | $K^+$ channel tetramerization domain containing 15 | C | IBID |
| M | GHM | *KCTD17* | $K^+$ channel tetramerization domain containing 17 | C | IBID |
| M | HMR | *KCTD3* | $K^+$ channel tetramerization domain containing 3 | C | IBID |
| M | HMR | *KCTD5* | $K^+$ channel tetramerization domain containing 5 | C | IBID |
| HR | DHM | *KCTD6* | $K^+$ channel tetramerization domain containing 6 | C | IBID |
| H | DHM | *KCTD7* | $K^+$ channel tetramerization domain containing 7 | C | IBID |
| R | DHM | *KCTD8* | $K^+$ channel tetramerization domain containing 8 | C | IBID |
| MR | DGHM | *KCTD9* | $K^+$ channel tetramerization domain containing 9 | C | IBID |
| R | M | *KLHL24* | Kelch-like 24 (*Drosophila*) | C | ICA |
| MR | D | *LCP1* | Lymphocyte cytosolic plastin 1 | C | $Ca^{2+}$ channel activity, $Ca^{2+}$ channel inhibitor activity, |
| H | H | *LOC729317* | Similar to voltage-dependent anion channel 2 | C | Voltage-gated ion-selective channel activity |
| DHMR | D | *MAT2A* | Methionine adenosyltransferase II, α | C | ICA, $K^+$ channel activity, $K^+$ channel inhibitor activity |
| R | D | *MCFD2* | Multiple coagulation factor deficiency 2 | C | $Ca^{2+}$ channel activity, $Ca^{2+}$ channel inhibitor activity |
| HMR | GHMR | *MCOLN1* | Mucolipin 1 | C | $K^+$ channel activity, cation channel activity |
| *Microarray* | DHMR | *MCOLN2* | Mucolipin 2 | C | ICA |
| RT-PCR | DHMR | MCOLN3 | Mucolipin 3 | C | ICA |
| *Microarray* | HR | *MS4A2* | Membrane-spanning 4-domains, subfamily A, member 2 | E | $Ca^{2+}$ channel activity |
| *Microarray* | D | *MYL7* | Myosin, light polypeptide 7, regulatory | C | $Ca^{2+}$ channel activity, $Ca^{2+}$ channel inhibitor activity |
| *Microarray* | D | *MYL9* | Myosin, light polypeptide 9, regulatory | C | IBID |
| R | R | *NID67* | Putative small membrane protein NID67 | C | ICA |

**TABLE 1 (continued)**

Ion channel genes expressed in the inner ear as of March 2007

| *Expressed* | *Genome* | *Gene symbol* | *Full name* | *GOE* | *GO annotation* |
|---|---|---|---|---|---|
| M | M | *NUDT9* | Nudix (nucleoside diphosphate linked moiety X)-type motif 9 | C | $Ca^{2+}$-activated cation channel activity |
| MR | DHMR | *P2RX2* | Purinergic receptor P2X, ligand-gated ion channel, 2 | E | $Ca^{2+}$ channel regulator activity, ligand-gated ICA |
| DM | DHMR | *P2RX3* | Purinergic receptor P2X, ligand-gated ion channel, 3 | E | ATP-gated cation channel activity |
| MR | BDHMR | *P2RX7* | Purinergic receptor P2X, ligand-gated ion channel, 7 | E | ICA |
| R | HMR | *P2RXL1* | Purinergic receptor P2X-like 1, orphan receptor | E | ICA |
| HMR | HMR | *PKD1* | Polycystic kidney disease 1 homolog | E | $Ca^{2+}$ channel activity, cation channel activity |
| *Microarray* | H | *PKD1L3* | Polycystic kidney disease 1-like 3 | C | ICA |
| HM | BDHM | *PKD2* | Polycystic kidney disease gene-2 | E | Ca channel activity, IMP cation channel activity |
| RT-PCR | HM | PKD2L1 | PKD2-like 1 | C | ICA |
| R | HMR | *PLLP* | Plasma membrane proteolipid | C | ICA |
| DR | D | *POLB* | Polymerase (DNA directed), β | C | $Na^+$ channel activity, $Na^+$ channel inhibitor activity |
| M | D | *RCN3* | Reticulocalbin 3, EF-hand Ca binding domain | C | $Ca^{2+}$ channel activity, $Ca^{2+}$ channel inhibitor activity |
| H | D | *RHOT1* | Ras homolog gene family, member T1 | C | IBID |
| R | GHMR | *RYR1* | Ryanodine receptor 1 (skeletal) | E | ICA, ryanodine-sensitive $Ca^{2+}$-release channel activity, $Ca_v$ channel activity |
| H | HM | *RYR2* | Ryanodine receptor 2 (cardiac) | E | ICA, ryanodine-sensitive $Ca^{2+}$-release channel activity, |
| R | GHM | *RYR3* | Ryanodine receptor 3 | E | IBID |
| M | HMR | *SCN10A* | $Na_v$, type X, α | E | ICA, $Na_v$ channel activity/complex |
| R | DGHMR | *SCN1A* | $Na_v$, type I, α | E | IBID |
| *Microarray* | HM | *SCN1B* | $Na_v$, type I, β | E | IBID |
| *RT-PCR* | DH | *SCN2A* | $Na_v$, type II, α | E | IBID |
| H | HMR | *SCN3A* | $Na_v$, type III, α | E | IBID |
| MR | BHMR | *SCN4B* | $Na_v$, type IV, β | E | IBID |
| RT-PCR | BGHMR | *SCN5A* | $Na_v$, type V, α | E | IBID |
| HR | HMR | *SCN7A* | $Na_v$, type VII, α | E | IBID |
| D | D | *SCN8AA* | $Na_v$, type VIII, α | E | IBID |
| *Microarray* | DHMR | *SCN9A* | $Na_v$, type IX, α | E | IBID |
| M | HMR | *SCNN1B* | $Na^+$ channel, nonvoltage-gated β1 | E | Amiloride-sensitive $Na^+$ channel activity |
| M | HM | *SHKBP1* | Sh3kbp1 binding protein 1 | C | $K_v$ channel activity/complex |
| HMR | D | *SLC25A12* | solute carrier family 25 (mitochondrial carrier, Aralar), member | C | $Ca^{2+}$ channel activity, $Ca^{2+}$ channel inhibitor activity |
| MR | MR | *SLC9A1* | Solute carrier family 9, member 1 | E | ICA |
| M | MR | *SLC9A2* | Solute carrier family 9, $Na^+/H^+$ exchanger, member 2 | E | ICA |
| DHMR | R | *SNAP25* | Synaptosomal-associated protein 25 | E | $K_v$ channel activity/complex |
| *Microarray* | D | *SPARC* | Secreted acidic cysteine rich glycoprotein | C | $Ca^{2+}$ channel activity, $Ca^{2+}$ channel inhibitor activity |
| *Microarray* | DHM | *STIM1* | Stromal interaction molecule 1 | E | $K_v$ channel activity, activation of store-operated $Ca^{2+}$ channel activity |

**TABLE 1 (continued)**

Ion channel genes expressed in the inner ear as of March 2007

| *Expressed* | *Genome* | *Gene symbol* | *Full name* | *GOE* | *GO annotation* |
|---|---|---|---|---|---|
| *Microarray* | H | *STX1B1* | Syntaxin 1B1 | E | Extracellular ligand-gated ICA, glutamate-gated ICA |
| DHMR | H | *TAX1BP3* | Tax1 (human T-cell leukemia virus type I) binding protein 3 | E | ICA |
| D | D | *TCTP* | Translationally controlled tumor protein | C | $Ca^{2+}$ channel activity, $Ca^{2+}$ channel inhibitor activity |
| H | D | *TLL1* | Tolloid-like 1 | E | ICA, $Ca^{2+}$ channel activity, $Ca^{2+}$ channel inhibitor activity |
| DM | HMR | *TMEM37* | Transmembrane protein 37 | C | $Ca_v$ channel activity/complex |
| HM | DGHMR | *TNFAIP1* | Tumor necrosis factor, α-induced protein 1 (endothelial) | C | $K_v$ channel activity/complex |
| D | DHMR | *TNNC1* | Troponin C, slow | C | $Ca^{2+}$ channel activity, $Ca^{2+}$ channel inhibitor activity |
| DM | BDHM | *TOMM40* | Translocase of outer mitochondrial membrane 40 homolog (yeast) | C | Voltage-gated ion-selective channel activity |
| MR | HMR | *TPCN1* | Two pore segment channel 1 | C | $Ca^{2+}$ channel activity, voltage-gated ICA |
| DHM | DHMR | *TRPA1* | Transient receptor potential A1 | E | $Ca^{2+}$ channel activity, ICA |
| MR | BGHMR | *TRPC1* | Transient receptor potential cation channel, subfamily C, member 1 | E | Store-operated $Ca^{2+}$ channel activity, $Ca^{2+}$ channel activity, ICA |
| RT-PCR | BM | TRPC2 | TRPC, member 2 | C | IBID |
| R | BHMR | *TRPC3* | transient receptor potential cation channel, subfamily C, member 3 | E | IBID |
| R | BHMR | *TRPC4* | Transient receptor potential cation channel, subfamily C, member 4 | E | IBID |
| *MPSS* | HM | *TRPM1* | Transient receptor potential cation channel, subfamily M, member 1 | E | ICA |
| HMR | HM | *TRPM3* | Transient receptor potential cation channel, subfamily M, member 3 | C | $Ca^{2+}$ channel activity, ICA |
| M | HMR | *TRPM4* | Transient receptor potential cation channel, subfamily M, member 4 | E | $Ca^{2+}$ channel activity, cation channel activity, ICA |
| RT-PCR | HMR | TRPMS | TRPM, member 5 | C | IBID |
| HM | DHMR | *TRPM7* | Transient receptor potential-related protein, ChaK | E | $Ca^{2+}$ channel activity, cation channel activity |
| *RT-PCR* | DHMR | *TRPV1* | Transient receptor potential cation channel, subfamily V, member 1 | E | Ion channel activity |
| *MPSS* | BHMR | *TRPV2* | Transient receptor potential cation channel, subfamily V, member 2 | E | ICA |
| M | GHMR | *TRPV4* | Transient receptor potential cation channel, subfamily V, member 4 | E | ICA |
| *MPSS* | DHMR | *TRPV6* | Transient receptor potential cation channel, subfamily V, member 6 | E | ICA |
| R | M | *TTYH3* | Tweety homolog 3 (*Drosophila*) | E | ICA, $Cl^-$ channel activity |
| HMR | BGHMR | *VDAC1* | Voltage-dependent anion channel 1 | C | Voltage-gated ion-selective channel/porin activity |

**TABLE 1 (continued)**

Ion channel genes expressed in the inner ear as of March 2007

| *Expressed* | *Genome* | *Gene symbol* | *Full name* | *GOE* | *GO annotation* |
|---|---|---|---|---|---|
| HM | BDGHMR | *VDAC2* | Voltage-dependent anion channel 2 | E | IBID |
| MR | BGHMR | *VDAC3* | Voltage-dependent anion channel 3 | E | Voltage-gated anion channel porin activity |
| DMR | HMR | *VGCNL1* | Voltage-gated channel like 1 | E | IBID |
| D | D | *zgc:110679* | zgc:110679 | C | $Ca^{2+}$ channel activity, $Ca^{2+}$ channel inhibitor activity |
| D | D | *zgc:110771* | zgc:110771 | C | IBID |
| D | D | *zgc:123271* | zgc:123271 | C | $K^+$ channel activity |
| D | D | *zgc:91851* | zgc:91851 | C | $Ca^{2+}$ channel activity, $Ca^{2+}$ channel inhibitor activity |
| D | D | *zgc:92169* | zgc:92169 | C | IBID |
| HMR | H | *ZIC2* | Zic family member 2, odd-paired homolog, *Drosophila* | C | $K_v$ channel activity/complex |

Ion channel transcripts presented in column 1 were initially identified in cDNA libraries made from human cochlea (H), mouse organ of Corti and mouse inner ear (M), rat vestibule (R), and zebrafish inner ear (D). Additional transcripts included were identified in mouse and rat microarrays, by MPSS of mouse inner ear, and by RT-PCR experiments. The *Genome* column presents the organisms whose genomic profiles were screened and found to contain specific genes annotated as having ion channel activity; the *GOE* column stands for Gene Ontology Evidence, with E and C referring to experimental and computational evidence, respectively; the *GO Annotation* column presents channel-related gene ontology annotations. We note that GO annotations are sometimes contradicted by experimental evidence. *B Bos taurus, D Danio rerio, G Gallus gallus, H Homo sapiens, M Mus musculus, R Rattus norvegicus, ICA* ion channel activity, *IBID* the same.

and supplementary materials along with their GenBank accession number or sequence.

## Ion channel isoforms of the inner ear

Table 2 lists ion channel transcripts identified in both the human fetal cochlear and mouse organ of Corti libraries using our EST mapping technique. Almost half of the voltage-gated and mechanosensitive ion channels are represented as splice variants not yet annotated in either human or mouse genome builds. Some of these variants are annotated as putative genes by UniGene. For example, novel *TRPM3* ESTs available in GenBank are grouped in the Mm.131943 UniGene cluster, along with known isoforms of this gene, although, novel human ESTs belong to the Hs.50319 cluster annotated as "transcribed locus, cDNA DKFZp761C1418". However, neither of the mouse or human novel *TRPM3* ESTs (Fig. 4a) was annotated in the UCSC genome browser, latest NCBI builds of human and mouse genomes, or SWISS-PROT, TrEMBL, GenBank mRNA, or RefSeq. Many cDNA library artifacts could potentially lead to identification of nonexisting splice variants. Thus, our confidence in novel isoforms is based on multiple EST–EST and organism-to-organism alignments. Thirty-seven human cochlear ESTs (28 unique clones) and 30 mouse clones are aligned to unannotated regions downstream of ion channel genes in human and mouse genomes. Among these novel transcripts, all novel isoforms identified by us correspond to novel or known isoforms of another organism (human cochlea vs mouse organ of Corti) (Fig. 4a–d). For several genes, an unannotated gene variant in mouse aligns to a known or predicted variant in humans (Fig. 4c,d, others not shown). For several other genes, an unannotated human gene variant aligns to a known mouse gene (Fig. 4b, others not shown). In addition, we have identified shorter than currently annotated versions of genes, such as those of *KCTD12/Kctd12* (not shown) and *KCNMA1* (Figs. 3, 4c). All ion channel genes listed in Table 2 are expressed also in the brain and other organs. Ion channel genes identified in the inner ear from the cDNA libraries analyzed are listed in the supplementary materials along with their GenBank accession number or sequence.

## Detection and characterization of expression of computationally predicted ion channel gene candidates

RT-PCR experiments using RNA derived from various mouse tissues, including inner ear, confirm the expression of some of the predicted "novel" gene isoforms listed in Table 2. Expression of *Otor* (otoraplin) illustrates cochlear-specific expression of a gene for comparison, while equivalent expression levels of *Gapdh* were observed in all tissues, in agreement with statistical analysis of EST libraries (see Supplementary Material). Figure 3 illustrates that for both *Trpm3* and *Kcna2*, transcripts of both the previously reported "known" isoforms and the newly identified longer isoforms are present in

**TABLE 2**

Ion channel genes reliably identified in both human cochlear and mouse Organ of Corti cDNA libraries

| *Symbol, aliases* | *Ion, gating mechanism, [GO evidence]* | *Cellular function, known or proposed* | *Phenotype when mutated* | *Gene variants, this study* |
|---|---|---|---|---|
| *CACNA1G* $Ca_{v3.1}$ | $Ca^{2+}$, voltage-gated; [E] | $Ca^{2+}$ signaling, pacemaking functions | Migraine, episodic vertigo | 1, known |
| *CLCN3 CLC3, ClC-3* | $Cl^-$, voltage- gated; [E] | Regulation of cell volume, stabilization of cell membrane potential, regulation of excitability | Neurodegeneration, dev. retardation, blindness, motor coordination deficit, sp. hyperlocomotion | 1, known; 1, novel-longer |
| *CLIC4 H1, huH1, p64H1* | $Cl^-$, voltage-gated; [E] | Cell volume, membrane potential, pH and electrolyte maintenance | Unknown | 1 known |
| *FXYD6 Php* | $Cl^-$, unknown' [C] | Muscle contraction | Unknown | 1 known |
| *GRIA4 GLUR4* | $K^+$, ligand-gated [E] | Synaptic targeting | Schizophrenia | 1 known |
| *KCNA2* $K_{v1.2}$ | $K^+$, voltage-gated; [E] | Repolarization after an action potential | Unknown | 1 novel-longer |
| *KCNJ10* $K_{ir4.1}$ | $K^+$, voltage-gated, inward rectifying [E] | Prolonged action potentials, endolymph regulation | Deafness | 1 known |
| *KCNJ16* $K_{ir5.1}$ | $K^+$, voltage-gated, inward rectifying [C] | Regulation of fluid and PH balance | Unknown | 1 known |
| *KCNK1* $K_{2p1.1}$ | $K^+$, voltage-gated, inward rectifying [E] | Resting membrane potential | Unknown | 1 known |
| *KCNMA1* $K_{Ca1.1}$ BK-α MaxiK Slo-α | $K^+$, voltage-gated, large conductance; [E] | Repolarization; fast, Ca-activated K current | Hearing loss; Brain-dysfunction: abnormal eye-blink reflex, locomotion, motor coordination | 1 novel in mouse (shorter) |
| *KCTD12* T1- $K_v$ | $K^+$, voltage-gated; [C] | Cell growth | Unknown | 1 known, 2 novel |
| *TRPM3 LTRPC3, MLSN2* | Cations, $Ca^{2+}$, tension-gated; [C] | $Ca^{2+}$ homeostasis | Unknown | 1 novel-longer |
| *VDAC1 PORIN* | $A^-$, voltage-gated; [E] | Mitochondrial maintenance | Macrosomia, psycho-motor retardation | 1 known |
| *VDAC2 VDAC6* | $A^-$, voltage-gated; [E] | Mitochondrial maintenance | Unknown | 1 known |

GO evidence codes are given in square brackets as: E, if experimental evidence of ion channel activity is referenced in Gene ontology resources for at least one vertebrate organism; *C*, if ion channel activity is inferred computationally.

mouse brain and cochlea, despite the fact that in the EST-based analysis of transcript frequencies, only the longer *Kcna2* isoform was observed in the ear. In both cases, sequencing PCR products shows that both "novel" isoforms appear to be 3′ UTR variants and do not alter the coding sequence of the genes. Expression of the longer isoform of *Trpm3* is also observed in kidney and liver by RT-PCR, although not detected by EST sequencing (see Supplementary Material). Expression of both *Trpm3* isoforms, in all tissues examined, is detected by additional PCR amplification (data not shown), suggesting *Trpm3* is expressed at lower levels in these other organs. Our statistical analysis also shows significantly higher expression of the shorter (known) *Trpm3* isoform in the ear vs brain, liver, and kidney (see Supplementary Material). Increased PCR cycles reveal expression of both *Kcna2* isoforms in kidney and liver, but not in the reference RNA (data not shown).

The new isoform of *Kcnma1* identified by our method is a smaller transcript than that previously reported in mouse. It is predicted to encode a protein with a slightly altered carboxyl terminus, changing the final eight amino acids from RDKQ*NRKEMVYR* to RDKQ*KYVQEERL*. Interestingly, the PCR primers chosen to amplify the known variant of mouse *Kcnma1* amplify a slightly longer product from cochlea as compared to the other tissues. Sequencing of the PCR products revealed that the band observed in the cochlea is 29 bp longer than the other bands due to alternative splicing and inclusion of a small exon. The resulting frameshift creates a longer carboxyl terminus of the protein, replacing the final eight amino acids with RDKQ*NATRMTRMGQAEKKWFTDEPDNAYPRNIQIKPMSTHMANQINQYKSTSSLIPPIREVEDEC*. Similar isoforms were reported previously in rat cochlea (Langer et al. 2003), and while this manuscript was under review, Beisel et al. (2007) reported the same variants in mouse inner ear validating our findings. Our sequences have since been submitted to GenBank (accession numbers EF490432 and EF473653). No other relevant mRNA sequence is available in the GenBank for mouse, and the *M. musculus* genome is not properly annotated.

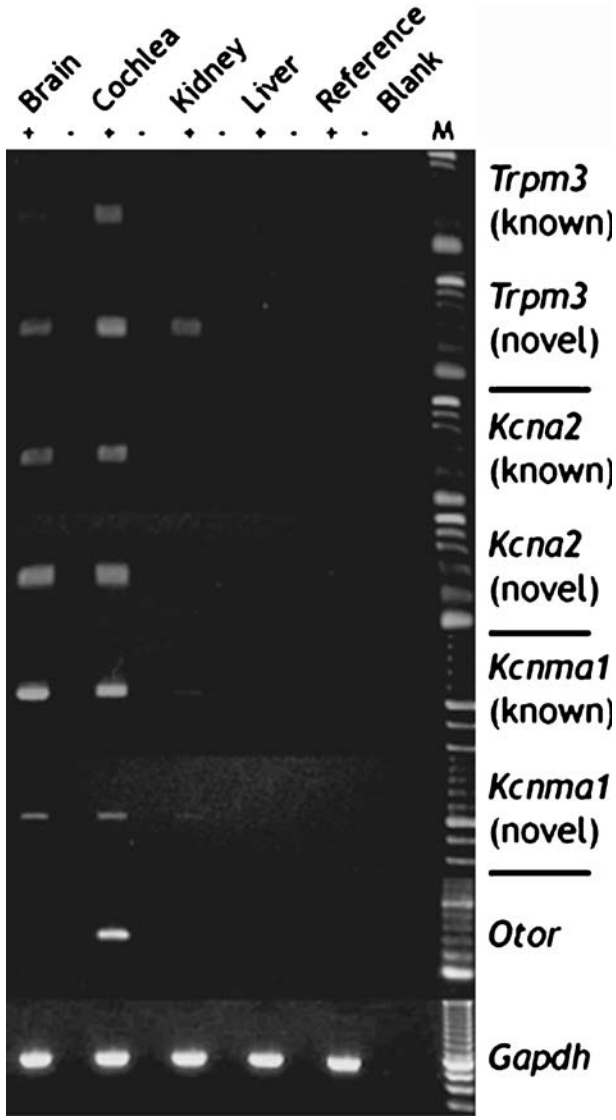


**FIG. 3.** RT-PCR products illustrate that both the "known" and "novel" splice variants of *Trpm3, Kcna2,* and *Kcnma1* can be detected in mouse RNAs derived from various tissues. Control genes included *Otor,* found only in inner ear, and *Gapdh,* which is ubiquitous in these tissues. The presence or absence of reverse transcriptase enzyme in the RT reaction is indicated by +/–, thus, controlling for genomic contamination. *Blank* indicates no sample. M is a 100-bp marker.

## DISCUSSION

Computational procedures were developed to identify genes of particular function by mapping the entire genome and applied to ESTs from a selected collection of inner ear cDNA libraries. We presented all ion channel genes including their possible splice variants identified by high-throughput methods in human, mouse, rat, and zebrafish, but focused on genes reproducibly found in human cochlea and mouse inner ear. Obviously, there are natural fluctuations in gene expression in the cell, and the accuracy of expressed gene counts improves as more data become available. While the majority of our analyses produce the same results as UniGene or other gene-indexing tools, a significant difference lies in our ability to identify novel transcript variants. Our results are necessarily biased toward the 3′ end of genes because the cDNA libraries analyzed were oligo d(T) primed and not full length. Even with this

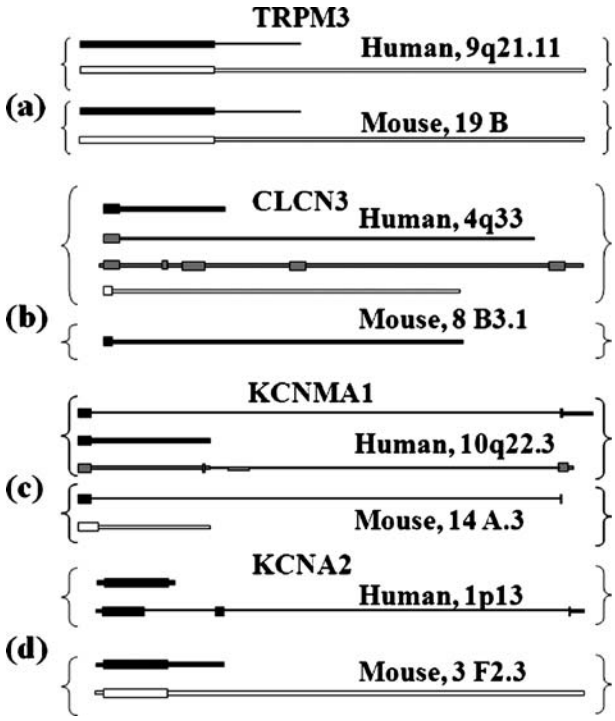


**FIG. 4.** Schematic representation of human and mouse ion channel gene isoforms aligned to the human genome. Each schematic shows only the most 3′ exon(s) and/or the 3′ UTR. **a** Human and mouse ESTs align to each other and to a region about 4,000 nt downstream of *TRPM3*. The last exon of the known gene and downstream regions of human and mouse gene aligned to the human genome are shown. **b** Novel *CLCN3* splice variant, predicted for the human cochlea based on EST analysis of the human fetal cochlear library, aligns to the known *Clcn3* mouse gene. AceView and ECgene predictions are also shown (*light blue*). **c** 3′ end of *KCNMA1* gene. A shorter UTR isoform is predicted for mouse. **d** The novel *Kcna2* splice variant aligns to a human RefSeq mRNA BC043564 annotated as *KCNA2*. *Black* Verified genes (RefSeq, GenBank, UniProt, UCSC), *gray* variants predicted by Ensemble, AceView, Vega or ECgene, *White (black contour)* indicates gene variants predicted in this work based on EST and polyadenylation signal analysis, *wide rectangle* coding sequence, *narrow rectangle* 3′ UTRs, *solid line* introns, *broken line* regions of poor alignment or uncertain sequence connection. Note that our predictions are only for 3′ regions.

limitation, we have identified and experimentally confirmed several novel variants among genes expressed in the inner ear. By using the EST mapping technique, we find that nearly half the ion channel genes identified in both the human cochlear and mouse organ of Corti cDNA libraries have previously unannotated variants. It is reasonable to expect that alternative transcripts differ in their expression patterns (Yura et al. 2006).

## Limitations of the method

The existing inner ear EST datasets are not representative solely of inner ear receptor cells, as they comprise only about 1% of the inner ear. While these data are expected to capture any genes with the highest level of expression, limitations are still set by the types and number of datasets screened. For example, we are limiting ourselves to some 15 genes when examining only ion channels reliably expressed in both human and mouse inner ear EST collections. If we look at ion channel transcripts available in cDNA libraries of at least two vertebrate organisms (human, mouse, rat, zebrafish), the gene count increases to 60. If we select ion channel genes identified in either human cochlea or mouse organ of Corti, the list of ion channel genes increases to 83 (78 if we exclude potential genomic contaminations). After addition of all other available cDNA libraries of the inner ear from human, mouse, rat, and zebrafish, the number of ion channel genes increases to 197.

As with any experimental system, different techniques can yield different results. Some ion channel genes were not identified by EST-based methods despite strong experimental evidence of their presence in microarrays, MPSS, and RT-PCR studies. For example, we did not find 18 genes in any of the inner ear EST libraries of the 36 ion channel genes detected in a mouse hair cell microarray experiment (Rivolta et al. 2002). Furthermore, the expression of over 50 ion channel genes not found by EST-sequencing was detected in inner ear tissues by microarrays or MPSS. In addition, RT-PCR experiments revealed at least eleven ion channel genes that have never been identified in any existing inner ear cDNA libraries, nor detected by microarray and MPSS experiments. These are *KCNN1* (Nie et al. 2004), *KCNN2* (Matthews et al. 2005), *KCNQ2* and *KCNQ3* (Liang et al. 2006), *SCN2A* (Chabbert et al. 2003), *SCN5A* (Wooltorton et al. 2007), TRPC2, TRPM5, PKD2L1, MCOLN3 (Cuajungco et al. 2007), and *TRPV1* (Peters et al. 2007). Thus, EST-based methods might miss up to 20% of expressed genes even if the library size is close to 20,000 sequences. Obviously, low abundant genes may easily be lost in tag sampling unless the sample is large enough, and in MPSS, the tag is placed close to the 3′ end (Wang et al. 2005; Chen and Rattray 2006). Microarrays detect only those genes that are present on the DNA chip, although their reliability is expected to improve with increased coverage of all possible gene splice variants. Genes with statistically significant levels of expression in microarray experiments may represent less than 70% of all actually expressed genes (Draghici et al. 2006). We find also that RT-PCR may give false negative results, possibly because of varied expression during different developmental stages or different experimental conditions (e.g., due to RT-PCR inhibitors).

In the following discussion, we relate only some of our findings to the experimental inner ear literature because a comprehensive review of all the findings is beyond the scope of this paper.

## Synopsis of differential gene expression in the inner ear

Our data show that $K^+$ channels have the highest expression levels in the ear, followed by nonselective cation channels, and then by $Ca^{2+}$ and nonselective anion channels, which are expressed at the lowest levels. We observed a wider variety of voltage-gated channels than purely ligand-gated channels. Amiloride-sensitive cation channels, for example, are expressed in low numbers in both mouse and rat inner ear cDNA libraries, but are not detected in the human cochlea by EST analysis.

## K+ channels

Cells of the inner ear differ from most other signal-conducting cells in their elevated use of $K^+$ ions, highly concentrated in the endolymph that baths the apical regions of the sensory cells. Thus, $K^+$ channels have a significant role in regulating $K^+$ ion movement from the cells of the stria vascularis through the sensory cells where voltage and ligand-gated channels fine-tune the transduced signal. Thus, there is a host of different $K^+$ channel types.

The large conductance $Ca^{2+}$-activated $K^+$ channel ($K_{Ca1.1}$, also called BK or MaxiK), has been studied extensively in the inner ear. The BK channel contributes to frequency tuning in hair cells of nonmammalian vertebrates, whereas in mammals, recent findings in BK knockout mice reveal no hearing loss, but a decreased sensitivity to noise (Fettiplace and Crawford 1980; Crawford and Fettiplace 1981; Lewis and Hudspeth 1983; Fuchs and Evans 1988; Fuchs et al. 1988; Ramanathan et al. 1999; Pyott et al. 2007). Despite these differences, a gene that encodes the α-subunit, *KCNMA1*, contains multiple splice variants in both mammalian and nonmammalian hair cells (Navaratnam et al. 1997; Rosenblatt et al. 1997; Jones et al. 1998; Ramanathan et al. 1999; Langer et al. 2003; Beisel et al. 2007). However, the mammalian variants are not annotated in the mouse and rat genomes. Only one *KCNMA1* gene variant was detected by mapping of the human and mouse ESTs, a shorter isoform annotated in human as transcript variant II (NM_002247) and previously unannotated in mouse. A short isoform of a *KCNMA*-like gene in zebrafish was also identified (data not shown). A second *KCNMA1* transcript variant was revealed fortuitously upon PCR amplification of the "known" transcript from mouse tissues. Orthologs of all of the above *KCNMA1* transcript variants are reported in mouse (Butler et al. 1993; Beisel et al. 2007) and rat cochlea (Langer et al. 2003), along with several other alternative splice variants of *KCNMA1*. These transcript variants, however, were not annotated in the mouse and rat genomes nor were corresponding mouse mRNA sequences available in NCBI's Entrez databases before our submissions (accession numbers EF490432 and EF473653).

A few members of the various Kv subfamilies were reported experimentally for the inner ear. These include Kv1.2 (*KCNA2*), which was recently immunolocalized to the vestibular dark cells and sensory epithelium of both human and mouse vestibular tissues; Kv1.4 and Kv1.5 were absent (Hotchkiss et al. 2005). We identified *KCNA2* in both human and mouse, and Kvβ1.2, also reported by Hotchkiss et al. (2005), in the larger mouse dataset. *KCNA2* is present as an extended isoform in both datasets. Our analysis suggests that the new isoform has a longer UTR than the canonical form, potentially with a previously unrecognized role in translational regulation. *KCNB1*, found in the mouse dataset was identified previously by PCR in *X. laevis* inner ear (Varela-Ramirez et al. 1998), while *KCNC1* was immunolocalized previously in mouse spiral ganglion (Adamson et al. 2002) and developing chick cochlear ganglion and hair cells (Zhou et al. 2001). Absent from cDNA libraries was Kv4.2 (*KCND2*), which is found in chick and mouse cochlear ganglion and hair cells (Adamson et al. 2002; Rivolta et al. 2002; Sokolowski et al. 2004).

Potassium currents through inward rectifier $K^+$ channels contribute to hearing. Loss of expression of a representative of this two-transmembrane (2 TM) domain subfamily ($K_{ir}$), *KCNJ10*, causes deafness by abolishing the endocochlear potential (Wangemann et al. 2004). We observed expression of *KCNJ10* in both human cochlea and mouse inner ear, while *KCNJ16* was expressed in all human and mouse datasets. RT-PCR experiments showed *KCNJ10* present in guinea pig cochlea from ED3 to adulthood (Jin et al. 2006).

*KCNK* members were detected in several datasets. This $K^+$ selective leak channel passes larger outward than inward currents due to open channel rectification (Plant et al. 2005). *TWIK-1* (*KCNK1*) and *TWIK-2* (*KCNK6*) were found previously in the rodent inner ear using RT-PCR and immunolocalized to the strial and dark cells of the cochlea and vestibule, respectively (Nicolas et al. 2003; Mhatre et al. 2004). *KCNK6* is a positional candidate for DFNA4, a form of autosomal dominant nonsyndromic hereditary hearing loss. *TREK-1* (*KCNK2*) was detected previously in rat cochlea using RT-PCR and immunolocalized to both sensory and supporting cells (Kanjhan et al. 2004).

We identified gene variants of a protein that contains an amino terminal similar to those found in Shaker channels. *KCTD12* is an intronless gene that appears to have predominant fetal expression in the human cochlea (Resendes et al. 2004). Three splice variants of *KCTD12* were identified in both human and mouse datasets, with the longest transcript being the most abundant. These splice variants differ in the UTR, which has several polyadenylation signals. The molecular role of this gene in the inner ear remains unknown, despite being a statistically significant differentiator of ear libraries vs all other tissues. *KCTD12* is annotated as a gene with "voltage-gated $K^+$ channel activity" [its GO evidence code is "inferred from electronic annotation" (IEA)] in human, mouse, and zebrafish genomes. This gene family encodes proteins with motifs akin to the tetramerization (T1) domain of Kv channels. This domain regulates α subunit initiation assembly and biophysical properties (Li et al. 1992; Bixby et al. 1999; Zerangue et al. 2000; Quirk and Reinhart 2001; Yi et al. 2001) and interacts with β-subunits (Gulbis et al. 2000) that regulate α function and surface expression (Accili et al. 1997a, b; Bahring et al. 2001; Lazaroff et al. 2002). However, any similarity to a functional ion channel needs additional verification.

## $Ca^{2+}$ and $Na^+$ channels

Our data suggest that $Ca^{2+}$ and $Na^{2+}$ channels are expressed in relatively lower numbers in the inner ear. High voltage-activated channel subunits *CACNA1E* (Cav1.2, α1C) and *CACNA1D* (Cav1.3, α1D) (L type) are present in both mouse and rat, while several other subunits such as the intermediate voltage-activated *CACNA1E* (R-type channel; Cav2.3) were detected only in mouse. The L-type $Ca^{2+}$ channel is found in the hair cells of various mammalian and nonmammalian inner ears (Kollmar et al. 1997; Ramakrishnan et al. 2002; Bao et al. 2003; Michna et al. 2003; Dou et al. 2004; Liang et al. 2004; Roche et al. 2005), where its loss in the cochlea leads to deafness (Platzer et al. 2000). The R-type $Ca^{2+}$ channel (*CACNA1E*, Cav2.3, α1E) was localized to the outer hair cells and their underlying nerves in guinea pig (Green et al. 1996; Layton et al. 2005), whereas in mouse, it appears in multiple structures during development (e.g., spiral bundle and efferent fibers), but only at the base of the outer hair cells in adult (Waka et al. 2003). We identified only one $Ca^{2+}$ channel gene, coding for the low voltage-gated, T-type, α1G subunit (*CACNA1G*), in both human and mouse libraries. $I_{CaT}$ is found briefly during different stages of development in the cochlear ganglion and hair cells of the chick (Jimenez et al. 1997; Sokolowski 2006; Levic et al. submitted). Both human and mouse libraries used in the present study are from fetal and late neonatal stages, respectively, suggesting a reason for detection in these libraries. $Ca^{2+}$ channel subunits α1A (P/Q type) and α1B (L and N type) were found in a rat vestibular library (Roche et al. 2005), while P and Q types were reported in recordings from chick embryonic cochlear ganglion cells (Jimenez et al. 1997). Other $Ca^{2+}$ channel subunits, for which there was no strong and consistent amplification, such α1S, β2, and γ, were never found in any of the available inner ear cDNA libraries, nor detected by microarray experiments.

Voltage-gated $Na^+$ channel genes (*SCN*) detected included *SCN1A*, *3A*, *7A*, *10A*, *8AA*, *1B*, and *4B*. $Na^+$ channels appear to have only a transitory role in some species. They are expressed in rat outer hair cells on postnatal days 0–9, but expressed barely by day 18 (Oliver et al. 1997). In mouse inner hair cells, $Na^+$ current (potential candidate Nav1.7; *SCN9A*) is present as early as ED16.5 and disappears by the onset of hearing on postnatal day 12 (Marcotti et al. 2003). In comparison, spiral ganglion cells of adult mouse are immunoreactive for Nav1.2 (*SCN2A*) and Nav1.6 (*SCN8A*) (Hossain et al. 2005). Previously, *SCN2A* and *SCNA3A* were suggested as candidate genes for deafness at the DFNA16 locus; the evidence, however, is inconclusive (Kasai et al. 2001). In addition, both Nav1.2 (*SCN2A*) and Nav1.6 (*SCN8A*) were identified using single cell RT-PCR on rat utricular hair cells (Chabbert et al. 2003), while Nav1.5 (*SCN5A*) was reported recently using electrophysiology, RT-PCR, and immunolocalization (Wooltorton et al. 2007).

## TRP channels

The molecular basis of mechanotransduction in hair cells of the inner ear remains to be elucidated fully (Clapham 2003; Kung 2005). *TRPA1* channels were initially thought to be the holy grail of auditory transduction channels in mammals (Corey et al. 2004), as another member of this superfamily, *TRPN1*, had been discovered previously in the hair cells of zebrafish (Sidi et al. 2003). More recent studies in mammals reported contradictory evidence for *TRPA1* (Bautista et al. 2006), although a candidate may still be found among this superfamily (see Cuajungco et al. 2007 for review). Our data show that three sets of ESTs from the human dataset align to orthologous *TRP* genes in mouse: *TRPM3*, *TRPM7*, and *TRPC4A*. Several *TRPM3* ESTs align to a region about 4,000 nucleotides (nt) downstream of the human *TRPM3* gene and about 2,000 nt downstream of the mouse *TRPM3* gene, suggesting the presence of a longer 3′ UTR than previously described. *TRPM3* has 24 exons spanning over 500,000 nucleotides in both human and mouse genomes. Multiple alterna-

tively spliced transcripts were predicted for *TRPM3*, and it seems likely that various *TRPM3* isoforms might be expressed in the inner ear. *TRPM3* splice variants detected by this analysis are not hearing-unique, as ESTs from eye, brain, kidney, and other tissues co-align with ESTs from the inner ear. Our RT-PCR experiments revealed that the predicted "novel" variant of *TRPM3* is, indeed, present in relevant tissues. *TRPV1* channel not revealed by high-throughput experiments was identified previously by PCR (Kitahara et al. 2005). *TRPV2* was identified recently by MPSS analysis of mouse inner ear (Peters et al. 2007).

### Anion channels

Nonselective anion channels were expressed at the lowest levels. In the inner ear, Cl– channels are found both in sensory and non-sensory tissues (Marcus and Marcus 1989; Wangemann and Marcus 1992; Ando and Takeuchi 2000). One of the novel human Cl– channel variants identified in this work, *CLCN3*, has a longer 3′ UTR than the classical variant and aligns to the known isoform of mouse *CLCN3*. Analysis of expressed human sequences from the GenBank database shows that the longer isoform is not cochlea specific. Other members identified include *CLIC5* and *CLCNKA* and *CLCNKB*. *CLIC5* is found in hair cell stereocilia, its loss leads to hearing deficiency in mouse knockouts (Gagnon et al. 2006). The latter two are functionally expressed in non-sensory tissues of the inner ear and the kidney (Ando and Takeuchi 2000; Hebert 2003; Maehara et al. 2003; Qu et al. 2006), although, there is some evidence for expression of the Clc-K1 channel (*CLCNKA*) in the outer hair cells (Kawasaki et al. 2000). Loss of hearing is associated with Bartter's syndrome type IV, which is the result of mutations in a β subunit, barttin (*BSND*), a protein that gives rise to significant Cl– channel activity when coexpressed with either *CLCNKA* or *CLCNKB* (Birkenhager et al. 2001; Estevez et al. 2001; Hebert 2003).

### Ligand-gated channels

Acid-sensing channels are potential candidates for mechanosensitive transduction in the stereocilia of the inner ear (Corey 2006). *ACCN1*, *2*, and *3* were present in rodent EST libraries. PCR studies revealed expression in the spiral ganglion and fibers of the mouse inner ear (Hildebrand et al. 2004). Functionally, *ACCN3* mouse knockouts show an increase in hearing thresholds from 2 to 3 months. However, there was no mutation of *ACCN3* in a family with *DFNB13* hearing loss, despite *ACCN3* mapping to the same chromosomal region as *DFNB13*. Deletions of other members, such as Asic2 (*ACCN2*), do not produce deafness in mice; although this mutation decreases spiral ganglion responses and susceptibility to noise (Peng et al. 2004). In comparison, Asic1b (*ACCN1*) channels are localized to the base of the stereocilia, but its function in the inner ear is not known (Ugawa et al. 2006).

We detected *CHRNA4*, *7*, *9*, and *10* in our study. A nicotinic sensitive ACh receptor that allows the influx of cations such as $Ca^{2+}$ mediates efferent inhibition in the outer hair cells (Fuchs and Murrow 1992; Hiel et al. 2000; Marcotti et al. 2004; Gomez-Casati et al. 2005). Both α9 (*CHRNA9*) and α10 (*CHRNA10*) subunits contribute to the formation of this heteromeric channel (Gomez-Casati et al. 2005; Plazas 2005). In our study, *CHRNA9* was detected in one normalized mouse cDNA library and previously by PCR (Nie et al. 2004). *CHRNA10* (nicotinic ACh receptor α10) was cloned previously from human ear and lymph tissue (Lustig et al. 2001).

*GABRA 1* and *2* of the gamma-aminobutyric acid (GABA) receptor genes were detected in rat and mouse databases, respectively. Sequences were absent in human cDNA libraries, whereas *GABRA1*, *2*, and *3* (GABA A α1, 2, and 3 subunits) were detected also in mouse microarrays. GABA receptors modulate efferents in the cochlea. Recent studies show that mutations in mice of GABA A α and β (*GABRB*) affect hearing differently (Maison et al. 2006). Those with mutations of the α1, 2, 6, and Δ subunits had normal hearing, whereas those with mutations of α5, β2, and 3 had elevated hearing thresholds suggestive of outer hair cell dysfunction.

We found only one glutamate receptor present in both human and mouse cDNA libraries, *GRIA4* (glutamate AMPA4). Excitatory postsynaptic currents are carried by AMPA receptor subunits (Glowatzki and Fuchs 2002) located postsynaptically on afferent terminals that innervate the inner hair cells. Immunogold labeling of AMPA receptor subunits in rat (GluR1-4), localize Glu2/3 and 4 postsynaptically on the afferent end terminals (Matsubara et al. 1996), whereas *in situ* hybridization studies in guinea pig suggest GluR2 (*GRIA2*) and 3 (*GRIA3*) mRNA expression in the spiral ganglion neurons and GluR4 (*GRIA4*) in the glia (Safieddine and Eybalin 1992). Presently, only immunoreactivity of Glu2 and 3 was observed in the inner ear (spiral ganglion) of mouse.

## CONCLUSION

Ion-channel-mediated signal transduction in the ear is very complex and requires investigation in the context of many other interactions and pathways. This analysis is a first step toward this goal, as it identifies

the most highly expressed and conserved ion channels in the ear, across multiple species. We show that large-scale experiments identify many but not all ion channels expressed in the inner ear. Moreover, we estimate that over half of the ion channel genome is expressed during the lifetime of the inner ear. All ion channels listed herein are present in other tissues (most often in the brain), illustrating the conservation of components among different "decision making processes" of cells receiving and transmitting electrical and mechanical signals.

Similarities between sequences of gene variants expressed in the ear are striking when comparing human and mouse, and new variants reported here and elsewhere are found in the analogous tissues of both species. These splice variants are observed in other tissues, often displaying a similar pattern of expression, suggesting the importance of transcriptional and translational regulation of ion channel genes in the inner ear, and analogously, in other organs and tissues as well. The main difference between the inner ear and other signal-conducting organs appears not to be in the specific variants of ion channel genes, but in their relative expression levels.

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