JARO 04: 24–40 (2002)
DOI: 10.1007/s10162-002-2058-2

JARO
Journal of the Association for Research in Otolaryngology

In Silico Analyses of Mouse Inner-Ear Transcripts

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Received: 27 December 2001; Accepted: 1 May 2002; Online publication: 20 June 2002

ABSTRACT

The development and function of the inner ear is complex requiring the correct and coordinated expression of many genes. The recent progress in the analyses of the human and other genomes has provided tools for identification of genes involved in hearing. As more and more nucleotide sequence information accumulates, experimental methods of molecular biology are rapidly being supplemented, and partially supplanted, by computational methods. In this study we present comprehensive *in silico* analyses of a cDNA library representing almost 1600 transcripts isolated from mouse inner ear. By mining the public databases we were able to rapidly and efficiently identify numerous transcripts likely to have a specific role in the auditory or vestibular function of the inner ear. Analyses revealed about 600 known genes and almost 100 inner-ear specific transcripts. Almost 50 of these are candidate genes for hearing impairment based on their chromosomal localization and inner-ear expression pattern. We describe a powerful approach to identify novel genes associated with hearing and vestibular function, further increasing our understanding of the molecular biology of the inner ear.

Keywords: mouse — inner ear — gene expression

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INTRODUCTION

The biomechanics of sound are well understood, but relatively little is known about the molecular basis of auditory function. The development and function of the inner ear is complex and requires correct and coordinated expression of thousands of genes. Studies on the molecular biology of the inner ear are hampered by the relative inaccessibility of the cochlea, by the limited number of cochlear cells, and by the inability to maintain many of these cell types in long-term cultures. Thus, the genetic approach is proving to be a powerful tool in revealing the molecular basis of hearing. Recent developments in the Human Genome Project and the progress made in other genomes has already resulted in identification of numerous genes involved in hearing. There are several comprehensive hearing-related databases providing a large amount of information on genes and hearing in an easily accessible form. Databases on hereditary hearing impairment in human and mice (Van Camp and Smith 2001; Zheng et al. 2001; http://www.ihr.mrc.ac.uk/hereditary/MutantsTable.Shtml), gene expression in the developing ear (Holme et al. 2001), human cochlear genes (http://hearing.bwh.harvard.edu/cochlearcdnalibrary.htm), and the proteins of inner ear (Thalman and Thalman 2001) have been established. In addition, microarray data from mouse inner-ear gene expression studies can be downloaded from the Internet (http://www.mgh.harvard.edu/depts/coreylab/index.html). These advances have made the identification of genes involved in hearing significantly more effective and feasible. Identifying such genes helps us understand the processes of both normal and impaired hearing.

Advances in the genetics of deafness have been reported in several recent reviews (Resendes et al. 2001; Steel and Kros 2001; Tekin et al. 2001).

Since the mammalian cochlea is largely conserved across different species, results from studies of animal models can be extrapolated to identify human genes involved in hearing. Due to practical considerations, such as size and anatomy of the ear, guinea pig and chinchilla have been favored species for inner-ear studies. However, recent advances in the characterization of the mouse genome and the wide use of the mouse as a model organism in other fields of biomedical research have made the laboratory mouse a primary model for hearing research. Natural mouse mutants and knockout mice with hearing loss provide powerful tools to dissect auditory function. Mouse mutants have been central in the discovery of a number of genes associated with hearing and deafness. Utilizing mouse models, more than 100 genes having an effect in the inner ear, either on its development or its function, have been identified. In addition to gene identification, mouse models enable further studies on deafness pathology and are crucial for therapeutic studies (Kiernan and Steel 2000; Probst and Camper 1999).

The development of human and mouse inner-ear cDNA libraries has provided a significant step toward the identification of genes involved in hearing. A cDNA library consists of a population of cDNA clones, each of which is synthesized from a single mRNA molecule expressed in a particular tissue. cDNA libraries can be used to create expressed sequence tag (EST) sequences which are short sequence reads. They represent a snapshot of genes expressed in a given tissue and/or at a given developmental stage. The amount of sequence in dbEST (database of ESTs, http://www.ncbi.nlm.nih.gov/dbEST/) has exploded over the last few years. About 10 million ESTs have been deposited so far, including 3.8 million human and 2.3 million mouse transcribed sequences. EST data have been applied to gene identification, comparative sequence analyses, comparative gene mapping, candidate disease gene identification, genome sequence annotation, microarray development, and transcription maps (Marra et al. 1999). Almost 15,000 human (Morton Fetal Cochlear cDNA library) and 4000 mouse (Soares Mouse NMIE cDNA library, RIKEN adult inner ear cDNA library) inner-ear ESTs are available from public databases. Skvorak and coworkers have reported their analyses on the human cochlear cDNA library demonstrating the high complexity of the library with many of the genes represented previously shown to be involved in hearing (Skvorak et al. 1999; Resendes et al. 2002). Updated information can be found in the Human Cochlear EST Database created by the authors (http://hearing.bwh.harvard.edu/cochlearcdnalibrary.Htm).

Although any gene expressed in the inner ear is of interest, genes with expression limited to the inner ear are of specific interest. Such genes are likely to be involved in the hearing/vestibular function or code for structural proteins characteristic and crucial for the inner ear. The discovery of tissue-specific transcripts results in novel information about the function of inner-ear and hearing disorders (Kubisch et al. 1999; Verpy et al. 1999, 2000). However, identification of such genes has been demanding, time consuming, and expensive. Traditionally, databases have been utilized to supplement experimental data, thus making gene identification more efficient. In this study we present *in silico* analyses of the Soares NMIE cDNA library. The EST sequences were analyzed against several databases, revealing interesting data about inner-ear gene expression. Five hundred eighty-eight known genes were represented in the library, of which at least 6 are associated with hearing loss. Based on their expression and chromosomal localization, 42 candidate genes for hearing loss were identified. In addition, the analyses revealed almost 100 inner-ear specific transcripts.

METHODS

Mouse inner-ear cDNA library

The Soares mouse NMIE cDNA library was constructed from 170 adult mouse inner ears by groups from the University of Iowa, USA, and the MRC UK Mouse Genome Centre and Mammalian Genetics Unit, Harwell, UK. As part of the IMAGE consortium (Integrated Molecular Analysis of Genomes and their Expression), 1536 clones from the library were arrayed. Of these clones 1644 ESTs (representing 1124 clones) were created and made publicly available. For some of the 1124 clones, ESTs were sequenced from both clone ends.

Databases

The EST sequences were downloaded through the UniGene Library Browser (http://www.ncbi.nlm.nih.gov/UniGene/lib.cgi?ORG =MmLID =388). The EST sequences were then analyzed against several NCBI databases (UniGene: http://www.ncbi.nlm.nih.gov/UniGene/; LocusLink: http://www.ncbi.nlm.nih.gov/LocusLink; HomoloGene: http://www.ncbi.nlm.nih.gov/HomoloGene). In addition, each sequence was locally compared against GenBank (http://www.ncbi.nlm.nih.gov/Genbank/index.html) databases (nr, htgs, EST) using the BLAST algorithm (Altschul et al. 1997). Information on the

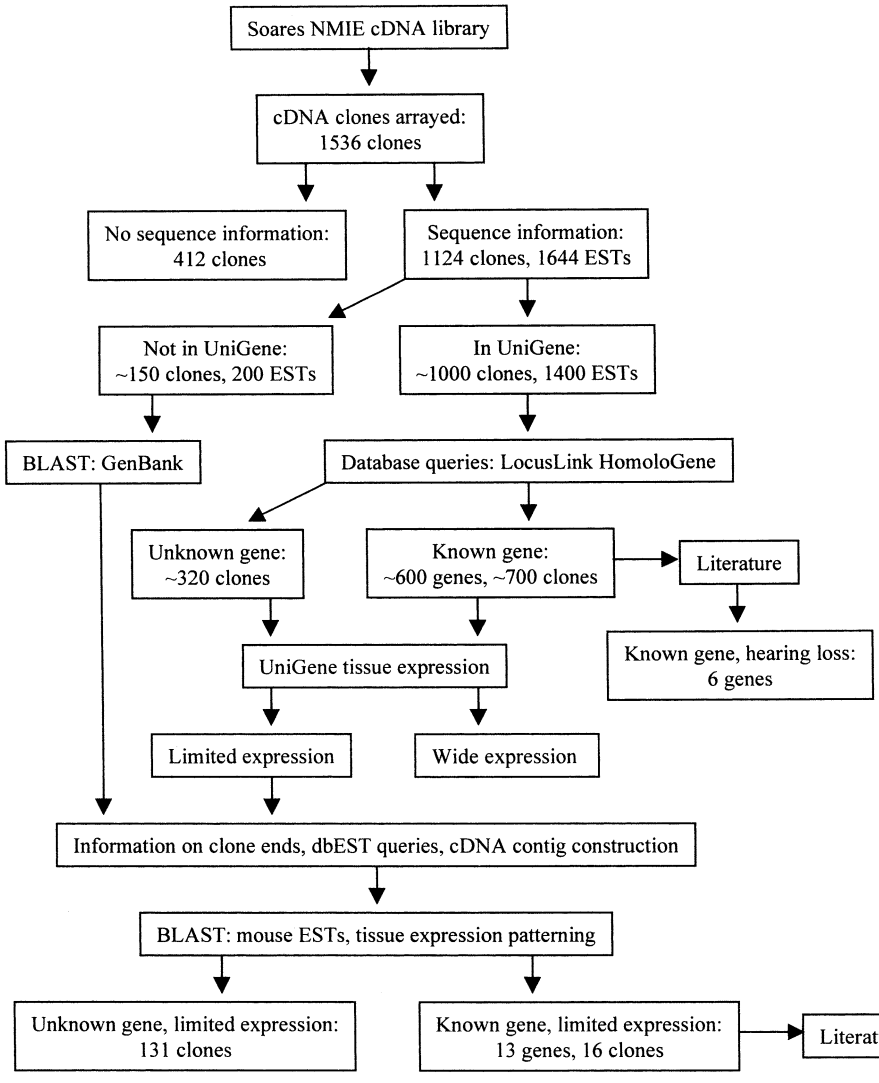

FIG. 1. A schematic picture of the *in silico* data mining.

known genes represented in the inner-ear EST pool was gathered from the literature, Jackson Laboratory Mouse Genome Informatics (www.informatics.jax.org/), OMIM, (Online Mendelian Inheritance in Man (www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM), and MRC inner-ear mutant table (www.ihr.mrc.ac.uk/hereditary/MutantsTable). The EST sequences were also locally compared against the Morton fetal human cochlear EST clones downloaded from UniGene (http://www.ncbi.nlm.nih.gov/UniGene/lib.cgi?ORG=HsLID = 200) using the BLAST algorithm (Altschul et al. 1997). In addition, a textword-based search was performed against the human cochlear cDNA library and EST database (http://hearing.bwh.harvard.edu/cochlearcdnalibrary.htm). All database queries were carried out between June and October 2001.

In silico tissue expression

The mouse inner-ear ESTs were analyzed for *in silico* expression pattern using UniGene information on the tissue distribution. UniGene clusters constructed from ESTs isolated from four or less different tissues (including inner ear) were selected, and the expression pattern was confirmed by BLAST searches against mouse dbEST. BLAST searches were also done for all mouse inner-ear ESTs not represented in UniGene. EST clustering and utilization of information from cDNA clone ends was used to analyze the *in silico* expression. The tissue expression of the known genes with limited *in silico* tissue expression was studied from the literature (Fig. 1).

Identification of positional candidate genes for hearing loss

The chromosomal localization of the mouse cDNAs was compared with the reported mouse hearing loss loci obtained from the Jackson Laboratory Mouse Genome Informatics database and from the MRC inner-ear mutant table. For the identification of putative human hearing loss genes, the human ortho-

TABLE 1

Known genes represented in the Scares NMIE inner-ear cDNA library[a]

Growth factors / embryogenesis

- 3-phosphoglycerate dehydrogenase
- AMY-1
- Bone morphogenetic protein 6
- Connective tissue growth factor
- Delta-tike homolog
- Dihydropyrimidinase-like 3
- Endothelial cell growth factor I
- Erythroid differentiation regulator
- Four and a half LIM domains 1
- Gene trap locus 3
- *Growth differentiation factor 1*
- Growth differentiation factor 10*
- Growth factor, erv1-like
- Growth hormone gene and promoter
- Insulin-like growth factor 2
- MAD homolog 4
- MAD homolog 5
- Mesoderm specific transcript
- MDM2 Binding protein
- *MyoD family inhibitor*
- Necdin
- Neural proliferation, differentiation and control gene 1
- Neurotrophin-1/B-cell stimulating factor-3*
- Palmitoyl-protein thioesterase
- Platelet derived growth factor, B polypeptide
- Pleckstrin homology-like domain, family A, member 3
- Protein kinase, cAMP dependent regulatory, type 1, alpha
- Secreted frizzled-related sequence protein I
- Semaphorin 3B

Cell cycle, growth, and aging

- AA27 mouse autoantigen P27 homolog
- ABL proto-oncogene tyrosine-protein kinase
- Anaphase-promoting complex subunit 7
- B-cell translocation gene 2, anti-proliferative
- Budding uninhibited by benzimidazoles 3
- C-src tyrosine kinase
- CD59a antigen
- Cullin 4B*
- Cyclin-dependent kinase 9*
- Cyclin-dependent kinase inhibitor 1A
- Cyclin-G associated kinase
- Cyclin D2
- Cyclin D3
- Cyclin-dependent kinase 4
- Era-like 1
- Glypican 1
- Glypican 6
- Growth factor receptor-bound protein 10
- Kirsten rat sarcoma oncogene 2
- MIG2 gene*
- Mitogen-activated protein kinase 1
- Mitogen-activated protein kinase 3
- Mitogen-activated protein kinase kinase kinase 6
- LIM and senescent cell antigen-like domains 1*
- Osteoclast-specific 116-kDa V-ATPase subunit
- PA26-T2 nuclear protein*
- Phosphatidic acid phosphatase 2b
- Pituitary tumor-transforming 1
- Procollagen C-proteinase enhancer protein
- Protein phosphatase 2a, catalytic subunit, alpha isoform
- Ral guanine nucleotide dissociation stimulator
- RAN, member RAS oncogene family
- RAN binding protein 9
- Src homology 2 domain-containing transforming protein C1
- **Thyroid hormone receptor alpha**

Apoptosis/anti-apoptosis

- Apoptosis inhibitory protein 5
- Death-associated kinase 3
- Defender against cell death 1
- Fas-associated factor 1
- Peroxiredoxin 1
- Peroxiredoxin 2
- Peroxiredoxin 3
- Peroxiredoxin 4
- Programmed cell death 2 (zinc finger protein RP-8)

Cytoskeletal structural protein

- Actin related protein 2/3 complex, subunit 1B
- Actin, alpha 1, skeletal muscle
- Actin, gamma, cytoplasmic
- Actin-associated protein 2E4/kaptin
- Actin-related protein 11 homolog
- Actin-related protein 3*
- Actinin alpha 2 associated LIM protein
- Alpha actinin 4
- ARPC4*
- Beta-spectrin 2, non-erythrocytic
- Cartilage oligomeric matrix protein
- Drebrin-like
- Ena-vasodilator stimulated phosphoprotein
- Erythrocyte membrane protein band 4.1-like 2*
- Fibromodulin
- Gamma-tubulin complex protein 2
- Matrilin 4
- Microtubule associated testis specific serine
- Myosin 1c
- Myosin binding protein C*
- Myosin light chain, alkali, fast skeletal muscle
- Myozenin
- Nude gene*
- Paralemmin
- Pleckstrin homology, Sec7 and coiled/coil domains 2
- Small protein effector 1 of Cdc42
- Smoothelin
- T-complex protein 1, related sequence 1
- Titin immunoglobulin domain protein
- Tropomyosin 2, beta
- Tubulin alpha 1
- Tubulin beta chain*
- Tubulin beta-3 chain
- Tubulin gamma polypeptide-like
- WAS protein family, member 2*

Microtubule-associated motor proteins

- Dynactin 3
- Dynactin 4
- Dynein, cytoplasmic, intermediate chain 2
- Kinesin light chain 1
- Kinesin light chain 2
- Mitofilin motor protein*
- Rab6, kinesin-like
- T-complex testis expressed 1

Cell adhesion / cell-cell interactions

- Collagen, type XVI, alpha 1*
- Dennatopontin

TABLE 1. Continued

Dystroglycan 1
Emilin*
Endoglin
Fibronectin 1
Integrin linked kinase
Laminin B1 subunit 1
Lymphocyte antigen 6 complex, locus E
Nidogen 2
Platelet/endotlielial cell adhesion molecule
Plexin C1*
Procollagen type XII alpha 1*
Procollagen, type I, alpha 1
Procollagen, type I, alpha 2
Procollagen, type II, alpha 1
Procollagen, type V, alpha i Catenin alpha 1
Proprotein convertase subtilisin/kexin type 3/furin
Protein tyrosine phosphatase, non-receptor type substrate 1
Similar to Plexin BI

Calcium binding / cadherins
Annexin A6
Annexin A11
Cadherin 13
Cadherin, EGF LAG seven-pass G-type receptor 3
Calmodulin 2
Calsequestrin 1
Calsyntenin 1
Fibulin 1
FK506 binding protein 6
Ionized calcium binding adapter molecule 2
Low density lipoprotein receptor-related protein 1
Osteonectin
Protein disulfide isomerase-related protein*
Protocadherin 10
Protocadherin 13
Stromal cell derived factor 1
Stromal cell derived factor 2
Stromal cell derived factor 4
Thrombospondin*
Visinin-like 1

Ion transport/Ion channels
Calcium channel, voltage-dependent, gamma subunit 6
Calcium channel, voltage-dependent, L type, alpha 1C subunit
Calcium channel, voltage-dependent, N type, alpha 1B subunit
Chloride channel 6
Connexin 47
DRASIC, proton gated cation channel*
MJAM gene (similar to solute carrier family member 11)
Neuronatin
Proteolipid protein 2
Reduced in osteosclerosis transporter
Ryanodine receptor 1, skeletal muscle
Sodium/hydrogen exchanger 5*
Solute carrier family 2, member 4
Solute carrier family 20, member 1
Solute carrier family 25, member 17
Solute carrier family 25, member 3*
Solute carrier family 25, member 4
Solute carrier family 29, member 1
Solute carrier family 9, isoform 3, regulator 2
Solute carrier family 9, isoform 6*
Voltage-dependent anion channel 1
Voltage-dependent anion channel 2
Voltage-dependent anion channel 3

ATP binding/proteasome
ATPase subunit 6
ATPase, aminophospholipid transporter, class I, type 8A, member 1
ATPase, H+ transporting, lysosomal, beta 56/58 kDa, isoform 2
ATPase H+ transporting lysosomal, noncatalytic accessory protein 1
ATPase, H+ transporting, lysosomal, subunit 1
ATPase, Na+/K+ beta 3 polypeptide
ATP-binding cassette, sub-family G, member 2
DEAD/H box polypeptide 18 (H.sapiens)
Death-associated kinase 3
Dystrophia myotonica kinase B15
Janus kinase 3
LIM motif-containing protein kinase 2
Peroxisomal biogenesis factor 6*
Proteasome 26S subunit, ATPase 2
Proteasome 26S subunit, ATPase 3
Proteasome 26S subunit, ATPase, 4
Proteasome subunit, alpha type 1
Proteasome subunit, alpha type 6
Proteasome subunit, beta type 6
RAD54 like
RNA-dependent helicase P72*
Valyl-tRNA synthetase 2

Intracellular protein traffic/signaling
3-phosphoinositide-dependent protein kinase-1
Adaptor protein complex AP-1, beta 1 subunit
Adaptor protein complex AP-1, gamma 1 subunit
Adaptor-related protein complex AP-3, delta subunit
Adenylate cyclase 6
Adenylyl cyclase-associated CAP protein homolog 1
ADP-ribosylation factor 1
Alkaline phosphatase 2, liver
Alpha-soluble NSF attachment protein*
Calcium/calmodulin-dependent protein kinase type 1*
Clathrin heavy chain*
Ctathrin, light polypeptide
Coated vesicle membrane protein
Coatomer protein complex, subunit beta 2
Coatomer protein complex, subunit zeta I
Coatomer protein complex, subunit zeta 2
Fibroblast growth factor receptor I
HS1 binding protein
Inhibitor of the Dv1 and Axin complex
Integral membrane protein Tmp21-I
LIM domains containing 1
Lymphotoxin B receptor
Mitochondrial import receptor subunit TOM20 homolog*
Phosphoprotein enriched in astrocytes 15
Protein kinase C, mu
Protein kinase inhibitor, alpha
RAP2A*
Rho interacting protein 2
SEC61, alpha subunit
Secretory carrier membrane protein 2
Secretory carrier membrane protein 4
TNFRSF1A modulator*
Translocator of inner mitochondrial membrane 44
Ywhae
Ywhah
Ywhaq
Zinedin*

TABLE 1. Continued

Electron transport
- Aldo-keto reductase family 1, member A1
- Degenerative spermatocyte homolog
- Fzrl protein
- Heme oxygenase 2
- Hexose-6-phosphate dehydrogenase*
- Lysyl-tRNA synthetase*
- NADH dehydrogenase flavoprotein 1
- NADH-ubiquinone oxidoreductase B15 subunit*
- NADH-ubiquinone oxidoreductase chain 1
- NADH-ubiquinone oxidoreductase chain 4
- NADH-ubiquinone oxidoreductase NDUFS2 subunit

Succinate dehydrogenase*
- Thioredoxin, mitochondrial
- Thioredoxin-like
- Thioredoxin-like 2
- Ubiquinol-cytochromc-C reductase complex core protein*
- Ubiquitin carboxy-terminal hydrolase L1
- Ubiquitin conjugating enzyme 2e
- Ubiquitin specific protease 11*
- Ubiquitin specific protease 23
- Ubiquitin specific protease 5
- Ubiquitin-associated protein NAG20
- Ubiquitin-conjugating enzyme E2 variant 1
- Ubiquitin-conjugating enzyme E2G 2
- Ubiquitin-conjugating enzyme E21
- Ubiquitin-like 1 activating enzyme subunit 1
- Ubiquitin-like protein GDX

DNA binding/transcription
- Activating transcription factor 4
- Basic transcription factor 2,35 kD subunit*
- Bromodomain-containing 4
- Butyrate response factor 1
- C-terminal binding protein 2
- CCR4-NOT transcription complex, subunit 2*
- Deformed epidermal autoregulatory factor 1
- DNMT1 associated protein-1
- E2F-like transcriptional represser protein
- Forkhead box A2
- General control of ami no acid synthesis-like 2
- General transcription factor IIH, polypeptide 1
- Glioma-amplified sequence-41
- High mobility group nucleosomal binding domain 2
- Inhibitor of DNA binding 1
- Inhibitor of DNA binding 3
- Interferon regulatory factor 6
- Iroquois related homeobox 1
- ISL1 transcription factor LIM/homeodomain
- Kruppel-like factor 2
- Kruppel-like factor 3
- Methionine aminopeptidase
- Mrg2
- Mut S homolog 5
- Myocyte-specific enhancer factor 2D*
- NF-YC-like protein
- Nuclear receptor co-repressor 2
- Nuclear-encoded mitochondrial elongation factor G*
- Nuclear factor of activated T-cells 5
- Origin recognition complex subunit 3
- PCAF associated factor 65 beta*
- Peroxisome proliferator activated receptor binding protein
- Pituitary tumor-transforming 1
- Polymerase, gamma
- Prefoldin 5
- Putative transcription factor
- Regulatory factor X-associated protein
- Ring finger protein 10
- Ring finger protein 4
- Sex comb on midleg-like 1
- Serum response factor
- Signal transducer and activator of transcription 5A
- Signal transducer and activator of transcription 5B
- SMARCD3*
- Synovial sarcoma, translocated to X chromosome
- Testis expressed gene 189
- Transcription factor 12
- Transcriptional co-activator CRSP34*
- Transforming growth factor beta 1 induced transcript 4
- U5 small nuclear ribonucleoprotein 116 kDa
- Y box protein 1
- Zinc finger protein 148
- Zinc finger protein 228
- Zinc finger protein 289
- Zinc finger protein 43*
- Zinc finger protein 46
- Zinc finger protein HRX*
- *Zinc finger protein of the cerebellum 2*

RNA binding/ribosomal proteins
- Acidic ribosomal phosphoprotein PO
- Adenosine deaminase ADAR*
- B4GALT5*
- Eukaryotic initiation factor 4B*
- Eukaryotic translation elongation factor 1 alpha 1
- Eukaryotic translation elongation factor 2
- Eukaryotic translation initiation factor 2 alpha kinase 1
- Eukaryotic translation initiation factor 2 beta subunit*
- Eukaryotic translation initiation factor 4, gamma 2
- Eukaryotic translation initiation factor 4H*
- Eukaryotic translation initiation factor 5*
- Eukaryotic translation initiation factor 5a
- Mitochondrial ribosomal protein L2
- Mitochondrial ribosomal protein L3*
- Mitochondrial ribosomal protein S25
- Ribonuclease, RNase A family 4
- Ribosomal protein L10
- Ribosomal protein L13, mitochondrial
- Ribosomal protein L13a
- Ribosomal protein L21
- Ribosomal protein L22
- Ribosomal protein L3
- Ribosomal protein L7a
- Ribosomal protein L8
- Ribosomal protein L9
- Ribosomal protein PO, 60S acidic*
- Ribosomal protein S24 gene
- Ribosomal protein S3a
- Ribosomal protein S4, X-linked
- Ribosomal protein S5
- Ribosomal protein S6
- Ribosomal protein S9
- Ribosomal protein, mitochondrial, S10
- RNA-binding protein SiahBP
- Signal recognition particle 14 kDa
- Signal recognition particle receptor alpha subunit*
- Speckle-type POZ protein
- Translation factor suil homolog*

Others/unknowns
- 3-hydroxy-3-methylglutaryl-coenzyme A synthase 2
- 3-oxoacyl-CoA thiolase*
- 6-phosphogluconolactonase*

TABLE 1. Continued

8-oxoguanine DNA-glycosylase 1
A disintegrin and metalloproteinase domain 15
A disintegrin and metalloproteinase domain 17
Acid sphingomyelinase-like phosphodiesterase 3a
ADP-ribosylation-like 3
ADP-ribosylation factor-like protein 1
Alanyl (membrane) aminopeptidase
Aminolevulinic acid synthase 2, erythroid
Apolipoprotein D
Arginyl-tRNA synthetase*
ART3 gene
Atic*
BAI2*
Butyrate-induced transcript 1
C-type lectin, superfamily member 6
Carbonyl reductase 2
Carboxypeptidase E
Casein kinase II, beta subunit
Caseinolytic protease, ATP-dependent, proteolytic subunit
Cathepsin K
Ccth gene for chaperonin containing TCP-1 cta subunit
CGI-10*
CG1-69*
Cell division cycle 42 homolog
Colon cancer antigen 43*
COP-coated vesicle membrane P24 precursor*
COP9, subunit 4
Carnitine palmitoyltransferase 2
CYB5
Cystathione-beta-synthase*
Cysteine and histidine-rich protein
Cysteine-rich protein 2*
Cytochrome C oxidase polypeptide III
Cytochrome C oxidase, subunit I
Cytochrome C oxidase, subunit IV
Cytochrome P450 2J9
Deleted in polyposis 1
Dipeptidyl peptidase III*
Dlxin-1
DnaJ homolog, subfamily B, member 6
DOM-3 homolog Z
Dorsal protein 1*
Down syndrome critical region gene a
Ectonucleotide pyrophosphatase/phosphodiesterase 2
EH-domain containing 1
Enolase 2, gamma neuronal
Enolase 3, beta muscle
Epsin 2
Esterase 10
F-box protein FBL2*
Fatso
Fatty acid binding protein 5, epidermal
Fatty acid synthase
Feminization 1 a homolog
Ferritin heavy chain
Ferritin light chain 1
Fibrinogen/angiopoietin-related protein
Frizzled homolog 4*
Fumarylacetoacetate hydrolase
G protein gamma 3 linked gene
GAG related peptide
Galactose-1-phosphate uridyl transferase
Gene rich cluster, C8 gene
Germ cell-specific gene 1
Glucose regulated protein
Glutamatc oxaloacetate transaminase 1, soluble
Glutamate receptor subunit 3*
Glutathione peroxidase 1
Glutathione S-transferase like
Glutathione S-transferase, mu 1
Glutathione S-transferase, mu 2
Glyceraldehyde-3-phosphate dehydrogenase
Glycine aminotransferase precursor*
Glycogen synthase 3, brain
Golgi peripheral membrane protein p65
GP36b glycoprotein*
Guanosine diphosphate dissociation inhibitor 1
H1 histone family, member 2
H19
H2A histone family, member Y
H2A histone family, member Z
Heat shock protein 20-like protein
Heat shock protein, DNAJ-like 1
Heat shock protein, DNAJ-like 2
Hemoglobin, beta adult minor chain
Hemopoietic progenitor cell antigen CD34 precursor*
Hepatitis B virus X interacting protein*
Herpud1
Homer, neuronal immediate early gene, 2
House-keeping protein 1
Hoxal regulated gene
Hpall tiny fragments locus 9c
Hsc70t gene
HSPC142*
Hyaluronic acid-binding protein 4
Hydroxylacyl-Coenzyme A dehydrogenase
Immunosuperfamily protein B12
Inositol hexakisphosphate kinase*
Insulin-like growth factor binding protein 5
protease
Interferon alpha responsive gene
KE03 protein
Kelch-like ECH-associated protein 1
Lactate dehydrogenase 2, B chain
Laminin receptor 1
Latent TGF beta binding protein 4S*
Latexin
Lectin, galactose binding, soluble 8
Leucine aminopeptidase*
Leucine rich protein, B7 gene
LIM-domain protein LMP-1*
Lipin 2
Lipocalin 7
LL5 protein*
LRP16*
Lysozyme
Lysyl oxidase-like
Mage-d2 protein
Makorin RING zinc-finger protein 2
Makorin, ring finger protein, 1
Mannosidase 2, alpha B1 Maspin*
Maternally expressed gene 3
Matrix gamma-carboxyglutamate protein
Matrix metalloproteinase 2
Matrix metalloproteinase 9
Mdgl-1 protein
Membrane-bound transcription factor protease, site 1
Methionine adenosyltransferase II, alpha
Methionine adenosyltransferase II, beta*
Methyltransferase-like 1
MinK-like protein
Mitsugumin 29
MORF-related gene X

TABLE 1. Continued

MUF1*
Mulibrey nanism gene*
Multifunctional protein ADE2
Murr1
N-acetyl galactosaminidase, alpha
N-acetylneuraminic acid 9-phosphate synthetase
Nedd4 WW domain-binding protein 1
Nedd4 WW domain-binding protein 4
Neuronal pentraxin receptor
Neurotensin endopoptidase*
NG28 protein
Nischarin
Nuclear protein 95
Olfactomedin related ER localized protein
Ornithine decarboxylase antizyme
Ornithine decarboxylase antizyme inhibitor
Otoconin 90
Palate, lung, and nasal epithelium expressed transcript
Palmitoyl-protein thioesterase
Paraoxonase 3
Pax transcription activation domain interacting protein
Peptidylprolyl isomerase A
Peroxisomal delta3, delta2-enoyl-Coenzyme A isomerase
Peroxisomal farnesylated protein
Phenylalanyl-tRNA synthetase-like*
Phosphatidylethanolamine binding protein
Phosphatidylinositol-4-phosphate 5-kinase, type II, alpha
Phosphoglucomutase 1*
Phosphoglycerate mutase
Phospholipase A2, group V
Phosphotidylinositol transfer protein, beta
Phosphotyrosyl phosphatase activator*
PM5 precursor*
Poliovirus sensitivity
Proline-rich Gla polypcptide 2
Prolyl 4-hydroxylase, beta polypeptide
Properdin factor, complement
Prosaposin
Prostaglandin E receptor 1
Prostaglandin F2 receptor negative regulator*
Prostaglandin transporter PGT
Protein tyrosin phosphatase 4a2
Protein tyrosine phosphatase, receptor type, L
PTD002*
TD010
Pyruvate kinase 3
Rab acceptor 1
RalGDS-like protein 3
RAS-related C3 botulinum substrate 1
Repeat family 3 gene
RERE*
Sec24B*
Selenoprotein P, plasma, 1
Serine proteinase inhibitor, clade D, member 1
Serine protcinase inhibitor, clade H, member 1
SET translocation
Seven transmembrane domain protein*
Sialyltransferase 1
Sialyltransferase 4A
Sialyltransferase 5
Signal sequence receptor, delta
Silica-induced gene 81
Small GTPase Rah
SNERG-1 protein*
Sorting nexin 3
Spermidine synthase
Spinocerebellar ataxia 10 homolog
Spinster-like protein
SSRA translocon-associated protein, alpha*
Steroid 5 alpha-reductase 2 like
Tetratricopeptide repeat domain
THI homolog
TNF intracellular domain-interacting protein
Tousled-like kinase 2
Translationally regulated transcript
Transforming growth factor beta regulated gene 1
Translation factor suil homolog*
Translationally regulated transcript
Transthyretin
Tripartite motif protein 8
Tryptophanyl-tRNA synthetase
Tubby protein*
Tubby-like protein 3
Tumor cell suppression protein HTSI*
Unc119 homolog
Vesicular inhibitory amino acid transporter
von Hippel-Lindau disease gene
WD-repeat protein 6*
WSB-1
WW domain binding protein 1
XAP89 protein

[a]Genes that are known to be associated with hearing loss are shown in bold. Genes with limited *in silico* expression pattern are printed *in italic*. About 700 cDNA clones were found to represent almost 600 known genes. Known genes that were found to be highly similar to mouse inner-ear cDNA clones are followed by an asterisk (*). The genes have been divided into several groups. The authors emphasize that the grouping is rough and there is large overlap between different groups.

logs of mouse inner-ear transcripts were identified based on database searches. The chromosomal localization of these genes was then compared against reported human deafness loci found from the Hereditary Hearing Loss Homepage (http://dnalab-www.uia.ac.be/dnalab/hhh/) and NCBI Entrez Genome Map Viewer (http://www.ncbi.nlm.nih.gov/cgi-bin/Entrez/map_search). Transcripts localizing within 2 cM from the reported hearing loss locus or between DNA markers restricting a critical region were designated as positional candidates.

RESULTS

The mouse inner-ear cDNAs with available sequence information were computationally analyzed against several databases. About 700 out of the 1536 cDNA clones (46%) were found to represent 588 known genes (identical or highly similar to previously reported genes). A further 6% of the clones were found to be moderately or weakly similar to known gene, and about 320 (20%) cDNA clones were found to represent unknown genes/splice variants with no significant homology to coding sequences in GenBank. For 412 clones (27%) no sequence information

TABLE 2

Candidate genes for mouse hearing impairment[a]

Mutant name	Symbol	Chr	Positional candidates	UniGene
Tilted head	thd, ub	1 (59.9)	?, Unknown transcript	Mm.30012
Achondroplasia	cn	4 (55.4)	Carnitine palmitoyltransferase 2	Mm.29499
			Sex comb on midleg-like 1	Mm.18718
Pirouette	pi	5 (40)	Ribosomal protein L9	Mm.14244
Bronx waltzer	bv	5 (63)	Ring finger protein 10	Mm.30051
Sightless	Sig	6 (1.0)	Paraxonase 3	Mm.9122
			Procollagen, type 1, alpha 2	Mm.4482
Nijmenjen waltzer	nv	7 (4.2)	Dystrophia myotonica kinase, B15	Mm.6529
Nervous	nr	8 (8.0)	Fibroblast growth factor receptor 1	Mm.3157
			Voltage-dependent anion channel 3	Mm.133962
			Secreted frizzled-related sequence protein 1	Mm.3171
Modifier of deafwaddler	mdfw	10 (30.3)	Highly similar to LIM and senescent cell antigen-like domains 1	Mm.29097
Age-related hearing loss	Ahl	10 (31.5)	Highly similar to LIM and senescent cell antigen-like domains 1	Mm.29097
Cocked	co	11 (46.0)	Tryosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon polypeptide	Mm.3308
			Myosin Ic	Mm.25194
Muted	mu	13 (21.0)	Bone morphogenetic protein 6	Mm.3997
Purkinje cell degeneration	pcd	13 (37.0)	MAD homolog 5 (Drosophila), similar to transcription activator Smad1 (H. Sapiens)	Mm.33951
Head tilt	het	17 (4.1)	T-complex testis expressed 1	Mm.1948
Quaking	qk	17 (5.9)	T-complex protein 1, related sequence 1	Mm.6797
Dancer	Dc	19 (6)	G protein gamma 3 linked gene	Mm.15985
			Osteoclast-specific 116-kda V-ATPase subunit	Mm.19185

[a]Twenty mouse transcripts map to the vicinity of reported mouse hearing loss loci and should be considered as candidate genes for hearing impairment in mice based not only on their chromosomal localization but also on their inner-ear expression.

TABLE 3

Candidate genes for human hearing impairment

Hearing loss locus	UniGene	Mouse gene	Human ortholog	Human Chr locus
DFNA4	Mm.29618	?, highly similar to latent TGF beta binding protein 4	Latent TGF beta binding protein 4	19q13
	Mm.27499	?, highly similar to seven transmembrane domain protein	Seven transmembrane domain protein	19q13.1
DFNA7	Mm.28935	Dermatopontin	Dermatopontin	1q12-q23
DFNA23	Mm.29317	Actin-related protein 11 homologue	HARP11	14q21.1-q23.3
DFNA30	Mm.3616	Polymerase, gamma	Hypothetical protein FLJ10719	15q25
	Mm.4487	Alanyl (membrane) aminopeptidase	Alanyl (membrane) aminopeptidase	15q25-26
	Mm.25368	MJAM gene	Solute carrier family 21, membrane 11	15q26
DFNA32	Mm.3862	Insulin-like growth factor 2	Insulin-like growth factor 2	11p15.5
	Mm.14802	H19	H19	11p15.5
	Mm.28392	Deformed epidermal autoregulatory factor 1	Deformed epidermal autoregulatory factor 1	11p15.5
DFNB6	Mm.7524	Dystroglycan 1	Dystroglycan	3p21
	Mm.23018	?, weakly similar to protein phosphates 2c family (C. elegans)	?	3p21.1
	Mm.22330	Nischarin	KIAA0800 gene product	3p21.1
	Mm.24806	?, Unknown transcript	DKFZP564o243 protein	3p21.1
	Mm.4083	SEMA3B	SEMA3B	3p21.3
	Mm.1090	Glutathione peroxidase 1	Glutathione peroxidase 1	3p21.3
DFNB14	Mm.30010	Actin related protein 2/3 complex, subunit 1B	Actin related protein 2/3 complex, subunit 1B	7q22.1-q31.11
DFNB15	Mm.424	ATPase, Na+/K+ beta 3 polypeptide	ATP1B3, ATPase, Na+/K+ beta 3 polypeptide	3q22-q23
DFNB18	Mm.30097	Proteasome subunit, alpha type 1	Proteasome subunit, alpha type 1	11p15.1
DFNB28	Mm.100312	?, Unknown transcript	Hypothetical protein FLJ12242	22q12.2-q13.1
	Mm.641	Activating transcription factor 4	Activating transcription factor 4	22q13.1
	Mm.144089	Platelet derived growth factor, B polypeptide	Platelet derived growth factor, B polypeptide	22q13.1

[a]The 22 mouse transcripts and putative human orthologs considered as candidate genes for human hearing loss based on their localization and inner-ear expression. The human orthologs of almost 800 clones were identified and localised to a certain chromosome. Twenty-two mouse genes were found to be positional candidates for 10 different human hearing loss loci.

was provided by the IMAGE consortium. A list of the known genes identified from the mouse inner-ear EST database is provided in Table 1. A complete table containing IMAGE number, clone identification name, EST ID, UniGene cluster number, gene name, presence in the human cochlear cDNA library and EST database, mouse and human chromosomal loci, prevalence in the library, and in silico tissue distribution are included in the electronic supplement (http://dx.doi.org/10.007/s10162-002-2058-2).

Hearing loss genes represented in the Soares NMIE library

Of the clones representing known genes, several had previously been associated with hearing impairment:

Matrix gamma-carboxyglutamate protein (*MGP*). Keutel syndrome is caused by mutations in the *MGP* gene which is involved in regulation of extracellular matrix calcification. The syndrome is characterized by multiple pulmonary stenoses, abnormal cartilage calcification, and/or ossification and neural hearing loss (Keutel et al. l972; Munroe et al. 1999).

Procollagen, type 1, alpha 1 (*COL1A1*). COL1A1 is a major collagen of skin, tendon, and bone. Osteogenesis imperfecta results from mutations in *COL1A1* or *COL1A2*. Types III and IV of this mainly skeletal syndrome have been associated with hearing loss (Nicholls et al. 1991). Also, the *COL1A1* knockout mouse model is reported to have inner-ear malformations (Bohne and Harding 1997).

Procollagen, type II, alpha 1 (*COL2A1*). Like COL1A1, COL2A1 is also a major collagen of skin, tendon, and bone. Abnormalities of type II collagen are involved in sensorineural deafness accompanying hereditary disorders such as spondyloepiphyseal dysplasia congenita and Stickler syndrome. Type II collagen may also be the target of an autoimmune process in some cases of acquired bilateral progressive sensorineural hearing loss (Helfgott et al. 1991).

Solute carrier family 25, member 4 (*SLC25A4*). Progressive external ophthalmoplegia (PEO) with mitochondrial DNA deletions can be caused by mutations in the *SLC25A4* gene. Symptoms include myopathy, progressive external ophthalmoplegia, and abnormalities associated with deletions of mitochondrial DNA. Several patients with bilateral sensorineural hearing loss have also been reported (Kaukonen et al. 1999).

Thyroid hormone receptor alpha (*Tshr*). Development, maintenance, and function of the immune system are altered in mice homozygous for mutations in the *Tshr* gene. In addition, defective development of balancing behavior and cochlear abnormality with associated hearing loss have been found in these homozygotes (O'Malleye et al. 1995).

Tubby protein. Homozygotes for mutations in the *Tubby* gene are recognizable by increased body weight composed of excess adipose tissue. Also, *Tubby* -/- mice have combined retinal degeneration and progressive hearing loss. The hearing loss is due to degeneration of the organ of Corti and loss of afferent neurons (Ohlemiller et al. 1995).

Positional candidates for hearing impairment

More than 500 (33%) of the clones in the NMIE library could be localized to a certain mouse chromosome or chromosomal region by public database queries. The human orthologs of almost 800 clones (50%) could, in turn, be localized to a certain human chromosome. The comparison of chromosomal localization resulted in identification of several coding sequences as candidate genes for previously reported hearing loss loci based on their inner-ear expression and chromosomal localization. Analyses revealed 20 mouse genes that could be designated as positional candidates for 15 different mouse models of hearing loss. Of these, 18/20 cDNAs were known genes, one was a cDNA clone highly similar to human *LIMS1* gene and one represented an uncharacterized transcript (Table 2). An additional 22 mouse transcripts were identified to have a human ortholog mapping to a previously reported human hearing loss locus thus representing candidate genes for these disorders (Table 3).

In silico tissue expression

The mouse inner-ear ESTs were analyzed for *in silico* expression pattern. According to UniGene, 216 ESTs had an *in silico* expression pattern limited to four or less tissues (including inner ear), of which 63 ESTs were inner-ear specific. Secondary checking of the ESTs with BLAST queries against GenBank sequences revealed that 130/216 (60%) had a wider tissue expression than reported in the UniGene database and only 112 filled our criteria for limited tissue expression. Of the inner-ear-specific UniGene clusters, only 31/63 (49%) were found to be truly tissue specific. In addition, 151 inner-ear ESTs not represented in the UniGene database were found to have limited an *in silico* expression pattern. Of these, 111 had EST hits specifically to the inner-ear or embryonic tissues. Thus, only 31/142 (22%) of inner-ear-specific ESTs were identified from the UniGene database and 111/142 (78%) were identified by BLAST analyses against GenBank sequences. When EST clustering and information from clone ends was used to further analyze the expression pattern, 147/1536 cDNA clones (13% of those with sequence information) were found to have a restricted in silico tissue expression

pattern and 91 of these clones were inner-ear specific. Thirteen of 147 clones represented known genes (identical or highly similar) and 8 clones were found to have some level of similarity to known genes. Twenty-eight of these clones could be chromosomally localized by public database searches. The clones with limited in silico tissue expression can be identified from the electronic supplement: (http://dx.doi.org/10.007/s10162-002-2058-2).

Known genes with limited *in silico* tissue expression

The 13 known genes with restricted in silico tissue expression pattern were as follows:

Cadherin, EGF LAG seven-pass G-type receptor 3 (*CELSR3*). The *CELSR3* gene was identified from a brain cDNA library. Northern blot analyses have shown expression in several regions of rat brain (Nakayama et al. 1998). A dbEST query using the *CELSR3* cDNA found EST hits to the following mouse tissues: neuronal (8), inner ear (1), other (2).

Connexin 47 *(Cx47)*. *Cx47* is a neuronally expressed gap junction gene. *In situ* and Northern studies have shown neuronal-specific tissue expression (Teubner et al. 2001). A dbEST query using the mouse *Cx47* cDNA found EST hits to the following mouse tissues: neuronal (26), inner ear (2), other (2).

Germ cell-specific gene 1 *(GSG 1)*. Tanaka et al. (1994) identified the *GSG1* gene from a testis cDNA library and reported a germ-cell-specific tissue expression. A dbEST query using the *GSG1* cDNA found EST hits to the following mouse tissues: testis (28), inner ear (2), other (2).

Growth differentiation factor 1 *(GDF1)*. Northern analyses have detected two *GDF1* transcripts with embryonic- and neuronal-specific expression (Lee 1990, 1991). A dbEST query using the *GDF1* cDNA found EST hits to the following mouse tissues: neuronal (39), embryo (9), inner ear (1), other (1).

Leucine rich protein, B7 gene *(LRPB7)*. *LRPB7* has been reported to have several splice variants (Ansari–Lari et al. 1997, 1998). *LRPB7* has a 88% similarity at the nucleotide level to a *Rattus norvegicus* neuron-specific enolase gene. A dbEST query using the *LRPB7* cDNA found EST hits to the following mouse tissues: testis (10), neuronal (5), thymus (4), inner ear (2), embryo (1).

MyoD family inhibitor *(MDFI)*. MDFI is a myogenic represser inhibiting the transactivation activity of MyoD family members by masking their nuclear localization signals (Chen et al. 1996; Kraut 1997). A dbEST query using the *MDF1* cDNA found EST hits to the following mouse tissues: embryo (10), mammary (7), heart (2), inner ear (1), other (1).

Neuronal pentraxin receptor *(NPR)*. NPR is an integral cell membrane protein expressed in neuronal tissues. NPR is suggested to play a role in neuronal uptake or synapse formation and remodeling (Dodds et al. 1997; Kirkpatrick et al. 2000). A dbEST query using the *NPR* cDNA found EST hits to the following mouse tissues: neuronal (4), inner ear (2), other (2).

Otoconin 90 *(OC90)*. OC90 accounts for more than 90% of total mouse otoconial protein, and RT-PCR analyses have demonstrated expression in the developing mouse otocyst (Meyer et al. 1996; Wang et al. 1998). A dbEST query using the partial *OC90* cDNA found EST hits to the following mouse tissues: embryo (17), inner ear (4).

Protocadherin 10 *(PCDH10)*. PCDH10 is a cell–cell adhesion molecule, with expression specific to the nervous system (Hirano et al. 1999). A dbEST query using the *PCDH10* cDNA found EST hits to the following mouse tissues: neuronal (20, of which 12 from retina), mammary (3), embryo (2), inner ear (1),lung (1).

Reduced in osteosclerosis transporter *(Roct)*. Brady et al. (1999) identified this gene abundant in normal kidney. *In situ* studies show that *Roct* is also expressed in developing bone. A dbEST query using the *Roct* cDNA found EST hits to the following mouse tissues: kidney (35), neuronal (14), inner ear(1).

Vesicular inhibitory amino acid transporter (Viaat). Viaat is a neurotransmitter, responsible for the storage of GABA and/or glycine in synaptic vesicles (Sagne et al. 1997; Gasnier 2000). A dbEST query using the *Viaat* cDNA found EST hits to the following mouse tissues: neuronal (44), embryo (4), inner ear (2).

Visinin-like *(VSNL1)*. The visinin and visinin-like peptides represent a family of calcium-binding proteins highly expressed in the retina (Polymeropoulos et al. 1995). A dbEST query using the *VSNL1* cDNA found EST hits to the following mouse tissues: neuronal (18), inner ear (1), skin (1).

Zinc finger protein of cerebellum 2 *(Zic2)*. *Zic2* expression during mouse embryonic development is consistent with a major role in brain and distal limb development (Nagai et al. 1991). In humans, Northern blot analysis showed expression of *ZIC2* in fetal brain only. A dbEST query using the *ZIC2* cDNA found EST hits to the following mouse tissues: neuronal (20), embryo (4), inner ear (2), lung. (1).

DISCUSSION

Relatively little is known about the molecular basis of inner-ear function. Recent identification of deafness genes has revealed not only information about hear-

ing loss, but has provided information about normal hearing. One limitation of this gene-mutation-based research is that it focuses on individual genes in isolation. To overcome this, we decided to study a inner-ear cDNA library as a whole. Our aim was to identify genes and novel transcripts involved in the unique function of the inner ear.

The *in silico* analyses of the Scares mouse NMIE library resulted in the identification of almost 600 known genes expressed in the mouse inner ear. A complete list is provided in Table 1. Among these identified genes were several gene families of specific interest which are discussed in detail below.

Cadherins. Cadherins are calcium-dependent adhesive proteins that mediate neural cell-to-cell interactions, histogenesis, and cellular transformation. Cadherins, in particular protocadherins, are thought to be involved in synaptic sorting. Mutations in Cadherin 23 (*Cdh23*) and Protocadherin 15 (*Pcdh15*) have been found to be causative for hearing impairment and are hypothesized to be involved in the development or maintenance of the stereocilia bundles of hair cells (Ahmed et al. 2001; Alagramam et al. 2001; Bork et al. 2001). Neither *Cdh23* nor *Pcdh15* was found among the cDNA library transcripts, but three other cadherins were identified. Transcripts encoding for *Cdh13*, *Pcdh10*, and *Pcdh13* were represented among the mouse inner-ear cDNA clones. It is possible that these cadherins also play a role in the maintenance of stereocilia in the inner ear. Interestingly, one of the identified cadherins, Protocadherin 10, had a restricted tissue expression pattern suggesting that it is relatively specific to the retina. We postulate that *Pcdh10* has a function unique to the sensory epithelium on the basis of its tissue specificity in conjunction with its identification among the transcripts isolated from the inner-ear cDNA library.

Collagens. According to the ''MRC table of gene expression in the developing ear,'' 11 different collagen types are expressed in the inner ear (*1A1*, *1A2*, *2A1*, *3A1*, *4A1*, *4A3*, *4A4*, *4A5*, *4A6*, *11A1*, and *11A2*). Of these, only collagens *1A1*, *1A2*, and *2A1* were found among the mouse inner-ear cDNA clones. However, sequences representing collagens *5A1*, *12A1*, and *16A1* were also identified. Col12A1 and Col16A1 both belong to the FACIT subgroup of collagens that are associated with type I or II collagen fibrils and play a role in the interaction of these fibrils with other matrix components (Shaw and Olsen 1991). According to the literature, Col5A1 is present in a variety of tissues as a minor collagen component. Col5A1 closely resembles collagen 11A1 and both are suggested to have a role in the control of fibrillogenesis (Fichard et al. 1994). Mutations in *Col5A1* have been shown to be causative for Ehlers–Danlos syndrome (EDS), which is an inherited disorder of connective tissue that affects multiple organ systems. Hunter and co-workers reported a survey demonstrating hearing, speech, voice, and language difficulties in a large cohort of EDS patients (Hunter et al. 1998). Based on their presence among the transcripts isolated from the mouse inner ear, we postulate that collagens 5A1, 12A1, and 16A1 are novel collagens of the inner ear and that the hearing problems of EDS patients might be secondary to the underlying collagen 5A1 defect.

Cytoskeletal structural proteins. Converting mechanical movement into electric signals puts great demands on the structural proteins of the inner ear. Cochlear micromechanics consists of a complex mixture of cross-talk among basilar membrane vibration, tectoral membrane and stereocilia movement, outer hair cell motility, and mechanical support from supporting cells. Actin, tubulin, and intermediate filaments provide the structural basis for cell shape changes and make up the cytoskeleton within the organ of Corti (Dallos et al. 1996). A number of deafness-associated mutations have been found in genes encoding structural proteins. As expected, among the Soares NMIE transcripts were numerous genes encoding structural proteins including different actins, myosins, and tubulins (Table 1). Despite the unique demands of inner-ear micromechanics, no tissue-specific transcripts were identified, but the structural proteins of the inner ear seem to have a role which is shared with other tissues. Mutations in several structural proteins, including myosins 6, 7A, and 15, have been reported to cause hearing impairment (Weil et al. 1995, 1997; Liu et al. 1997; Mustapha et al. 1999; Melchionda et al. 2001). Among the deafness candidate genes identified (Tables 2 and 3) were three genes that encoded for structural proteins: actin-related protein 2/3 complex subunit 1B, actin-related protein 11 homolog, and myosin 1C. Their function, chromosomal localization to the vicinity of reported hearing loss loci, and their inner-ear expression are suggestive of potential deafness genes. Due to the phenotype reported in mice with mutations in other myosin genes, we postulate that myosin 1C is especially likely as a causative gene for the inner-ear defective *cocked* mouse.

Growth factors. Growth factors regulate many events during development of the ear. After the embryonic period, growth factors support cell survival and innervation of new sensory cells. Growth factors may have the therapeutic potential to protect and/or stimulate the replacement of damaged sensory hair cells, thereby assisting in alleviating hearing loss and vestibular dysfunction (Corwin et al. 1996; Oesterle and Hume 1999). It is also known that growth factors enhance spiral ganglion cell survival following deafness from ototoxic drugs or noise (Miller et al. 1997).

Several growth factors and genes involved in embryogenesis were identified from the mouse inner-ear cDNA sequences. Two of the growth/embryogenesis-related genes identified, growth differentiation factor 1 (*GDF1*) and MyoD family inhibitor (*MDFI*), were found to have limited *in silico* tissue expression. The Soares mouse inner-ear library was made from adult mouse inner ears; thus, it is likely that the growth factors identified have roles in the adult inner ear. We hypothesize that due to the role of the identified growth factors in cell survival, and their presence among the mouse inner-ear transcripts, some of these factors (or their antagonists) might act as therapeutic agents for sensory defects of the inner ear. Tissue-specific *GDF1* and *MYODI* are considered as especially good candidates for therapeutic studies on inner-ear defects.

Inner-ear-specific transcripts. We identified 13 known genes with limited *in silico* tissue expression from the mouse inner-ear cDNA library. Previous experimental studies on these genes confirmed our hypothesis of restricted tissue expression in all cases. This suggests that the inner-ear-specific transcripts encoding for unknown genes are truly tissue specific and of specific interest for hearing/vestibular researchers. Due to their highly restricted tissue expression, these unknown transcripts are likely to encode polypeptides with specific auditory or vestibular functions. Among the known genes with restricted tissue expression were several of high interest. Cadherins and connexins belong to groups of proteins previously associated with hearing impairment. We identified transcripts encoding for cadherin EGF LAG seven-pass G-type receptor 3 (*CELSR3*) and connexin 47 (*Cx47*), suggesting that these transcripts should be considered as candidate genes for hearing impairment based on their function and expression. Two growth factors, *GDF1* and *MDFI*, with restricted tissue expression, were identified and were discussed in more detail above. Although all the known genes with limited tissue expression are of certain interest, we think that genes encoding for Reduced in osteosclerosis transporter (*Roct*) and for Germ cell-specific gene 1 (*GSG1*) are the most exciting. As might be expected, the majority of the known genes with restricted tissue expression were highly expressed in neuronal tissues. However, the *in silico* EST pattern suggests that *Roct* expression is mainly limited to kidney and *GSG1* is highly specific to testis. *Roct* belongs to the organic ion transporter family. The function of this group is to eliminate endogenous and exogenous toxins and many of these genes are expressed in the adult kidney. In the adult mouse, expression of *Roct* is kidney specific, but some expression during embryogenesis has been reported in liver, bone, and neuronal tissues (Brady et al. 1999, Pavlova et al. 2000). The Soares NMIE library was made from adult mouse cochleas. We postulate that in addition to its role in kidney, *Roct* might also function as a highly tissue-specific organic ion transporter of the inner ear. *GSG1* is a developmentally controlled gene reported to be exclusively expressed in testis (Tanaka et al. 1994). The role of this transcript, selectively expressed in testis and inner ear, is unknown. Interestingly, Espin and myosin VIIa are actin-binding proteins of hair cell stereocilia and Sertoli cell–spermatid junctions. Espin expression is reported to be abundant only in testis and hair cells (Bartles et al. 1996; Zheng L et al. 2000). In addition to hair cells and testis, myosin VIIa is expressed in retina, lung, and kidney. All of these tissues share functional cilia (Wolfrum et al. 1998). Based on its restricted *in silico* expression pattern, restricted to testis and inner ear, it is tempting to speculate that *GSG1* has an actin-binding function in hair cell stereocilia. Future studies are required to reveal the extremely interesting functions of *Roct* and *GSG1* in the inner ear.

Candidate genes. One of the problems with genetic linkage studies of nonsyndromic deafness is that the phenotype between different individuals is often similar despite having different causative genes. Most linkage studies have been done with large deafness families where the disease gene can be expected to be shared between affected family members. This makes the linkage studies more efficient, but single families often result in a linked chromosomal region that is too large for positional cloning of disease genes. Often the best and/or only way to identify disease genes requires mutation analyses of genes previously mapped to the critical linkage region. However, the larger the region of interest the more positional candidate genes there are to be analyzed. Our *in silico* analyses have identified genes that make good candidates for hearing impairment (Tables 2 and 3). Among these candidate genes were several of specific interest. The human orthologs of the mouse *MJAM* gene (Solute carrier family 21, member 11) and ATPase beta 3 polypeptide (*ATP1B3*) map to the chromosomal regions previously linked with human hearing impairment. Also mouse transcripts encoding for COL1A2 and myosin 1C colocalize with mouse deafness loci. Members of all these groups have been previously shown to be causative for hearing loss. We postulate that these genes make strong candidates as deafness genes based not only on their chromosomal localization, but also on their inner-ear expression and function.

Although our studies resulted in the identification of several tissue-specific transcripts and revealed interesting information about the genes expressed in the inner ear, there are a couple of issues in the

methodology that one should be aware of. We postulate that the identification of transcripts expressed in the mouse inner ear will help us to understand the biology of this highly specialized organ. While this is true, the problem with transcript-based analyses is that these studies do not address levels of regulation that lie downstream of transcription. The level of a transcript does not necessarily correlate to the amount of polypeptide encoded by it. In addition, it is estimated that two-thirds of the approximately 20,000 genes expressed by a typical cell are expressed at low abundance (Zhang et al. 1997). By analyzing only 1536 clones, it is evident that no information on low-abundance transcripts was obtained. Despite the normalization of the Soares NMIE library, the cDNA clones analyzed are likely to represent only a relatively small portion of the more abundant inner-ear transcripts. The inner ear is composed of dozens of highly specialized cell types. By analyzing whole organ pools, the unique characteristics of all these different, highly specialized cells and a large amount of interesting data are lost. For example, oncomodulin and prestin are reported to be expressed selectively in outer hair cells (Sakaguchi et al. 1998; Zheng et al. 2000). Neither of these genes were identified among the mouse inner-ear transcripts. There are several possible reasons for this. Only 1536 clones were arrayed and only part of them sequenced. Although the level of prestin and oncomodulin transcripts would be high in outer hair cells, the overall level in inner ear might be relatively low. Alternatively, both genes might be represented in the library but were not picked when the library was arrayed or sequenced. To avoid these problems, organ of Corti and even hair-cell-specific cDNA libraries from the inner ear have been created (Crozet et al. 1997; Dulon et al. 1998; Harter et al. 1999; Zheng J et al. 2000). No doubt these libraries will provide additional information on the characteristics of these particular cell types. Unfortunately, no large-scale EST information is available from these cDNA libraries on the public databases. It would be of great advantage for hearing/vestibular research if publicly available sequence information were gathered from all inner-ear-related cDNA libraries, especially from those representing single cell types.

By *in silico* analyses of public databases we were able to rapidly and efficiently identify transcripts likely to have a specific role in the auditory or vestibular function of the inner ear. About 13% (147) of the Soares inner-ear cDNA clones with sequence information in the databases had restricted in silico tissue expression and almost 100 of them had an expression pattern limited only to the inner ear. It is likely that these transcripts represent novel genes and splice variants specific to the inner ear. When combining our data with gene prediction programs utilizing the mouse genomic sequence, it should be possible to identify several inner-ear-specific genes with no tedious ''wet-lab'' experiments needed. We have printed the Soares mouse inner-ear cDNA library onto a microarray, as the characteristics of this library make it highly suitable for inner-ear gene expression studies. Combining the information from *in silico* analyses with microarray experiments should greatly enhance microarray data analyses.

ACKNOWLEDGMENTS

We acknowledge the financial support from the Murdoch Childrens Research Institute, Finska Läkaresällskapet, Wilhelm och Else Stockmanns Stiftelse, the Garnett Passe and Rodney Williams Memorial Foundation, and J. & J. Calvert–Jones. H.-H. M. Dahl is a NHMRC Principal Research Fellow. Dr. M. de Silva and Dr. M. Hildebrand are thanked for their comments on the manuscript.

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