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Construction and characterization of a vestibular-specific cDNA library using T7-based RNA amplification

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Abstract Using a very small amount of inner-ear tissue, we constructed a human vestibular cDNA library by means of T7-based amplification of RNA. This library should allow us to identify genes likely to be involved in auditory and vestibular functions. Here we first report the characterization of the human vestibular cDNA library. Among 506 cDNA clones randomly selected from the vestibular cDNA library, DNA sequences of 301 cDNA clones were identical to those of genes of known function. Twenty-two cDNA clones were considered to be novel because they did not match any cDNA sequences in the public database. The information in our study will provide a valuable resource for identifying several novel genes underlying deafness disorders and vestibular dysfunction.

Key words Human vestibule · cDNA library · T7-based RNA amplification · Hearing loss · Vestibular dysfunction

Introduction

Analysis of the human genome at the molecular level has made it possible to identify genes responsible for hereditary deafness; to date investigators have cloned 28 genes associated with nonsyndromic deafness and dozens involved in deafness syndromes (multiple phenotypic disease).

Among several different approaches for identifying aberrant genes responsible for hearing disorders, the most common strategy has been the positional-candidate ap-

proach coupled with gene-mapping information and genetic linkage analysis, using selected groups of affected and unaffected individuals. Many genes, including *GJB2*, *TECTA*, and *OTOF*, have been identified in this way (Kelsell et al. 1997; Verhoeven et al. 1999; Yasunaga et al. 1999). Although this method is very effective for discovery of novel deafness-related genes, narrowing a candidate locus to a very small interval requires participation of relatively large families and comprehensive clinicopathological data. Another approach is based on specific expression patterns or functions in target organs. Inner-ear cDNA libraries have been constructed from multiple samples of fetal cochlear tissue to isolate genes associated with hearing (Robertson et al. 1994; Jacob et al. 1997). This approach has identified three genes responsible for deafness: *ATQ1*, *COCH*, and *OTOR* (Robertson et al. 1994, 1997, 2000; Skvorak et al. 1997). A large series of cochlea organ analysis has been performed, including more than 4000 human expressed sequence tags (ESTs) from the fetal cochlear library deposited in the public database (see <http://hearing.bwh.harvard.edu/cochlearcnlibrary.htm>). However, to date, no attempt has been made to characterize the expressed genes of the human vestibule representing a functionally essential compartment in the inner ear. Vestibular symptoms are clinically either sole complaints or sometimes associated with deafness. Among the cochleo-vestibular dysfunctions, Meniere's disease, which is defined as a clinically representative inner ear disorder accompanied by sensorineural hearing loss and vertigo, was dominantly inherited in some families (Fung et al. 2002). The importance of genetic factors in Meniere's disease has been suggested, but very few genes associated with vestibular dysfunction have been cloned. The *COCH* gene, which is predominantly expressed in the inner ear, is demonstrated to be responsible for one type of nonsyndromic hearing loss with progressive hearing loss and vestibular dysfunction (Robertson et al. 1998). In contrast to the recent progress in the discovery of genes responsible for hearing loss, genes related to vestibular symptoms are not yet fully understood. Although many similarities are observed in the general features of cochlea and vestibular end organs, there are consid-

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erable differences in these homologous organs, including their evolution, structure, and function.

Therefore, as an additional resource for investigating genes essential for hearing and equilibrium, we constructed a human vestibular cDNA library by means of T7-based amplification of RNA from a very small amount of adult inner-ear tissue (Luo et al. 1999), and here describe the detailed analytical results obtained from reverse transcriptase-polymerase chain reaction (RT-PCR) experiments. To our knowledge, this is the first study of the gene expression profiles of human vestibular tissue.

Materials and methods

RNA extraction and T7-based amplification

Tissue samples of an adult vestibule were obtained from a single patient during labyrinthectomy with prior written informed consent, and immediately stored at -80°C . Total RNA was extracted from each tissue with an RNeasy mini kit (Qiagen, Valencia, CA, USA). Because of the extremely small amount of vestibular total RNA available at nanogram-level quantities, we applied one cycle of T7-based RNA amplification after DNase I treatment of total RNA and subsequent cDNA synthesis, as described previously (Luo et al. 1999), with some modifications.

Construction and analysis of a vestibular cDNA library

For second-strand cDNA synthesis, a linker-primer that included an internal *Xho*I site (5'-AGTCTCGAGTCTAGTCGACGGCCAGTGAATTGTAATACGACTCACTATAGG GCGT₂₁-3') was used. After double-strand cDNA fragments of less than 200bp were removed by the use of CHROMA spin column-400 (CLONTECH, Palo Alto, CA, USA), a cDNA library was constructed using the cDNA Synthesis kit (Stratagene, LaJolla, CA, USA) according to the supplier's instructions. From recombinant clones, we randomly selected 506 clones and determined their DNA sequences. The nucleotide sequence of each clone was compared with those of cDNA sequences in the GenBank database of the National Center for Biotechnology Information as of October 2002. The clones were classified according to the entries in GenBank (Table 1).

Table 1. Classification of cDNA sequences from the vestibular tissue

Database match	<i>n</i> (%)
Number of clones sequenced	506
Known function genes	301 (59.5)
Repetitive sequences	139 (27.5)
Clones identical to reported ESTs	29 (5.7)
Novel ESTs	22 (4.3)
Mitochondrial DNA sequences	15 (3.0)

EST, Expressed sequence tag

RT-PCR analysis

From among the 506 EST sequences obtained from the vestibular cDNA library, we selected clones that showed no match to any cDNA sequences in the database. Twenty-two novel EST sequences were submitted to the Center for Information Biology and DNA Data Bank of Japan (DDBJ); their GenBank accession numbers are AB0728187, AB072818, AB077323, AB074042, AB076975, AB074038, AB074039, AB072821–AB072825, AB074046, AB07673, AB074133, AB074135, AB076955, AB076958, AB076959, AB076961, AB076962, and AB076966. To determine the tissue expression patterns of the selected ESTs, we examined them by semiquantitative RT-PCR analysis using one round of T7-based amplified cDNAs from one vestibular organ and eight organs (brain, heart, placenta, lung, liver, skeletal muscle, kidney, and pancreas). Human glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was used as a quantity control. Sets of primers corresponding to each gene were designed from nucleotide sequences of the vestibular cDNA clones obtained, and these sets were first tested on genomic DNA to test the PCR. Amplification was performed at an annealing temperature of 95°C for 2 min, followed by 30–35 cycles of 95°C for 30s, 55°C for 30s, and 72°C for 30s. The annealing temperature was adjusted according to the T_m of the primers used. PCR products were electrophoresed on a 2%–3% agarose/Ethidium bromide gel.

Results

Evaluation and functional categorization of the vestibular cDNA library

The cDNA library was constructed from membranous labyrinths (vestibule) from adult inner ear. Very careful microscopic dissection was performed to avoid contamination by surrounding bony, cartilaginous, and fibrous elements. We obtained approximately 2.7×10^4 plaques and performed a partial characterization using 506 randomly selected clones. The insert sizes of these cDNA clones ranged from 0.2 to 0.5kbp. Their DNA sequences were compared with sequences in the public database and classified as shown in Table 1. Of the 506 clones sequenced, 301 (222 independent clones), representing 59.5% of the total, were identical to sequences of genes whose functions were known; 29 (5.7%) were identical to archived EST sequences; 15 (3.0%) were identical to mitochondrial sequences; and 22 (4.3%) did not match any cDNA sequences in the public database. DNA sequences of all or part of the remaining 139 clones (27.5%) were repetitive elements. Genes corresponding to ESTs are summarized in Table 2. Comparison of our 222 independent vestibular genes of known functions with DNA sequences from a fetal cochlear library reported previously (Skvorak et al. 1999) revealed that 53 of these cDNAs were common to both studies (Table 2). The vestibular cDNA sequences that were identical to genes of known function were classified into 17 categories according to their functional

Table 2. The functional categories of known genes found in the vestibular cDNA library

Gene description	Occurrence	Accession no.	Identical to cochlear ESTs
Cell cycle/cell proliferation			
Apoptosis inhibitor survivin	1	HSU75285	
BBP-like protein 1 (BLP1)	1	AF353991	
bcl-1	2	HUMBCL1	
Cell cycle regulator protein p95	1	AF058696	
Chondroitin sulfate proteoglycan 2 (versican) (CSPG2)	1	NM004385	
WD repeat domain 7 (WDR7), transcript variant 1 (= TGF-beta resistance associated gene)	1	NM_015285	
Programmed cell death-2/RP8 homolog (PDCD2)	2	AL031259	
Cell surface markers/cell adhesion			
Integral transmembrane protein	1	ITM1	
Junctional adhesion molecule 3 (JAM3)	1	NM_032801	
Ligatin	2	AF159586	
Lysosomal-associated protein transmembrane 4 alpha (LAPTM4A)	1	NM_014713	
Progesterone membrane binding protein (PMBP)	1	NM_006320	
Pro-oncosis receptor inducing membrane injury gene	1	HSM800854	
Putative type 1a integral membrane protein	1	HSS171	
Retinoic acid receptor responder (tazarotene induced) 1 (RARRES1)	2	RARES1	
Similar to integral membrane protein 3	1	BC002424	
Tight junction protein 1	2	TJP1	
Transmembrane protein 1 (TMEM1)	1	XM009794	
Transmembrane protein BRI	1	AF152432	
Extracellular matrix			
Alpha-3 type IV collagen (COL4A3)	1	HUMCOL4A3X	
Collagen type IV alpha 5 (COL4A5)	1	U04520	+
Endoplasmic reticulum glycoprotein	1	NM_006816	
Cellular metabolism			
Amino adipate-semialdehyde dehydrogenase-phosphopantetheinyl transferase	1	AF151838	
Beta1-4 galactosyl transferase	1	AF038662	
Carbonic anhydrase II (CA2)	1	NM000067	+
CDP-diacylglycerol synthase (CDS)	1	HSU6008	
Cisplatin resistance associated (CRA)	1	AW00589	+
Cytochrome C oxidase subunit IV precursor (COX4) gene, encoding mitochondrial protein	1	AF017115	+
Cytosolic selenium-dependent glutathione peroxidase	1	HUMGLPEX	+
Diaphorase (NADH) cytochrome b-5 reductase, nuclear gene encoding mt protein,	1	DIA1B	
Dioxin inducible cytochrome 450	2	HSU56438	+
Ectonucleotide pyrophosphatase/phosphodiesterase 4	2	NM_014936	
Filamin (FLN) gene to glucose-6-phosphate dehydrogenase (G6PD)	1	HUMFLNG6PD	
FRA3B common fragile region diadenosine triphosphate hydrolase (FHIT)	1	AF020503	
Glutathione peroxidase 4 (CPX4)	1	GPX4	
Inducible 6-phosphofructo-2-kinase/fructose 2, 6-bisphos	1	AF056320	+
Lactate dehydrogenase B (LDHB)	2	LDHB	
p6=cytochrome c oxidase subunit VIc homolog/COSVIc/prostatic carcinoma upregulated	1	S82616	+
Phenylalkylamine binding protein	3	AF196969	
Prostaglandin D2 synthase gene, exon 7	1	HUMDS03	
Putative DNA dependent ATPase and helicase (ATAX)	1	HSU72938	
Pyruvate dehydrogenase E1-alpha subunit	1	HUMPDHE1B	+
Retinal short-chain dehydrogenase/reductase	1	AF126782	+
Rhodanese (=thiosulfate sulfurtransferase)	1	D87292	
Serine palmitoyl transferase (SPTL1)	1	AF286717	
Sphingomyelin phosphodiesterase	1	X52678	+
Stannin	5	AF286717	+
Thiopurine methyl transferase (TPMT)	3	HSTHSMT1	
TPI1 gene for thiosephosphate isomerase	1	HSTPI1G	
Unspliced mRNA for glutathione peroxidase	2	HSPEROXP	
Cytoskeletal proteins			
Actin-gamma 1	1	ACTG1	+
Alpha-actin	2	HSAPACT	
Beta-2-microglobulin	1	B2M	+
C3 (complement component) (C3) gene 3'-end	1	M63422	
Cytokelatin exon, delta-aminolevulinic synthase (erythroid)	1	HS884M20	
Lamin A/C	1	BC003162	
WAS protein family, member 2	1	AK025566	

Table 2. Continued

Gene description	Occurrence	Accession no.	Identical to cochlear ESTs
DNA replication and repair			
Topoisomerase II beta	1	U65315	+
X-ray repair complementing defective repair in Chinese hamster cells 3 (XRCC3)	1	XM_050297	
Serum/blood cell proteins			
Apolipoprotein cluster	1	HSALINT	+
B-cells—hypothetical protein	1	AA362439	
Beta-globin	2	AF007546	
hHa2 gene from circulating lymphocyte	1	X90761	
Ig gamma heavy chain, membrane-bound type, and secret type	1	D78345	
Immunoglobulin heavy chain variable region	1	HSA7320	
Immunoglobulin lamda	1	D88268	+
Neuronal			
Ceroid-lipofuscinosis, neural 3, juvenile (CLNS3)	1	NM000086	
Glioblastoma amplified secreted protein (GASP)	1	AF395824	
Highly similar to SPECTRIN beta chain, brain	1	AK023762	
NDRG family member 4 (NDRG4)	1	NM_022910	
Neuroxin III-alpha	3	AF099810	
Purkinje cell protein 2	1	S40022	
Sine oculis homeobox homolog 3 (<i>Drosophila</i>) (SIX3)	2	NM_005413	
Translation/protein synthesis			
28S ribosomal RNA	7	AF152922	
40S ribosomal protein S18	2	AL031228	+
60S ribosomal protein L13a	1	X56932	
60S ribosomal protein L15	1	AF279903	+
60S ribosomal protein L37A	1	HS495010	
Heterogeneous nuclear ribosomal protein11	1	HNRPA1	
Ribosomal proteon S6	1	HSRPS6G	+
Ribosomal protein L3	3	HUMRPL3A	+
Ribosomal protein L9	3	RPL9	+
Ribosomal protein L15	1	HS90L6	
Ribosomal protein L17	1	RPL17	+
Ribosomal protein L27	2	HUMRIBPROE	
Ribosomal protein L35a	1	RPL35A	+
Ribosomal protein L37	1	L11567	+
Ribosomal protein L41	1	AF026844	+
Ribosomal protein L44	1	RPL44	
Ribosomal protein S11	1	RPS11	+
Ribosomal protein S12	2	NM001016.1	+
Ribosomal protein S19	1	RP19	
Ribosomal protein S24	3	HSU12202	+
Ribosomal protein S25	1	RPS25	+
Ribosomal protein S27A (ubiquitin carboxyl extension protein)	1	RPS27A	+
Ribosomal protein S7	1	RPS7	+
Ribosomal protein S23	1	RPS23	+
Surf 3 gene for ribosomal protein L7A	2	HSSURF3	
TATA binding protein (TBP)-associated factor	1	TAF2G	
The gene for U2 small nuclear RNA-associated beta antigen	1	HS705D16	+
RNA processing			
Nucleolar protein family A, member 2 (H/ACA small nucleolar RNPs) (NOLA2)	1	NM_017838	
The alternatively spliced gene for the human orthologs of mouse QKI-7 and QKI-7B	1	HS51J12	
Transcription/nuclear proteins			
HIV Tat-SF1	2	U76992	
Homeobox protein NKX3.1	1	AF247704	+
Max	1	HSMAXG	
Nuclear protein family 3 similar to integral membrane	1	BC002424	
Nuclear transport factor 2	1	BC002348	
Transcription factor ETR103	1	HUMETR103	
Zinc-finger protein 147 (ZNF 147) (estrogen-responsive finger protein)	1	XM012593	
Kinase and phosphatase			
Eph-like receptor tyrosine kinase hEphB1b (EphB1)	2	AF037332	
PI-3 kinase	2	HSPI3KINK	+
PNP1 mRNA (thyrosine phosphatase)	1	HSPNP1	
Protein kinase inhibitor gamma (PKIG)	2	AF182032	
Protein phosphatase 1, gamma	1	L07395	
Protein phosphatase 1, catalytic subunit, alpha isoform	2	BC004482	+

Table 2. Continued

Gene description	Occurrence	Accession no.	Identical to cochlear ESTs
PTPL mRNA for protein tyrosine phosphatase	2	HSPTPL1	+
RYK = related to receptor tyrosine kinase from human hepatoma	1	S59184	
Similar to serine threonine kinase pim3	1	BC017083	
YSK1	1	D63780	
Proteolysis			
F-box only protein 7	3	XM_00994	
Polyubiquitin UbC	3	AB009010	
Proteosome (prosome, maropain) subunit, beta type 1	1	NM_002793	
Sorting nexin1A (SNX1)	2	AF065484	
Signaling molecules/growth factors/receptors			
Amyloid beta (A4) precursor protein (Alzheimer disease)	1	XM009710	+
Calmodulin 1	1	JO4046	+
Cytoplasmic interactor of beta 4 integrin P27BBP protein (ITBG4BP) gene, exon 4, 5, 6, 7	1	AF289541	
Diaphorase (NADH) (cytochrome b-5 reductase) (DIA1), nuclear gene encoding mt protein	1	NM007326	
Elongation factor 2	2	HSEF2MR	+
Glutamate receptor, metabotropic 1 (GRM1)	1	XM_054004	
Insulin-like growth factor 2 receptor (IGF2R)	1	XM604237	
Insulin-like growth factor binding protein 6 (IGFBP6)	1	HSA6952	
Metabotropic glutamate receptor 6 (mGluR6)	2	HSU82083	
Okadaic acid-inducible and cAMP-regulated phosphoprotein 19	1	AF084555	+
p0071 protein	1	HSP0071	
Solute carrier family 1 (glial high-affinity glutamate transporter), member3 (SLC1A3)	1	SLC1A3	
Testosterone-repressed prostate message 2 (TRPM-2) protein gene	1	M63379	
Thrombin receptor gene, exon1	1	AF054633	
Transmembrane receptor protein	1	HSTRECP	+
Uterin bom besin receptor	1	HSUBR	
Zona pellucida glycoprotein 2 (sperm receptor)	1	ZP2	
Channel protein			
Apamin-sensitive small-conductive calcium-activated potassium channel (hSK2) (KCNN2)	1	AF239613	
ATPase, H ⁺ transporting, lysosomal mRNA	1	ATP6C	
ATPase, Na ⁺ /K ⁺ transporting, alpha2 (+) polypeptide	2	ATP1A2	+
Potassium channel, subfamily K, member 6 (TWIK-2)(KCNK6)	1	KCNK6	
Vestibule-1 protein (mechanosensitive ion channel domain containing protein)	2	AB056652	
Sodium/myo-inositol cotransporter (SLC5A3)	1	AF027153	
Voltage-dependent anion channel 3 (VDAC3)	1	BC002456	
Voltage-dependent calcium channel beta2 subunit (CACNB2)	1	AF465485	
Miscellaneous			
40-kDa keratin intermediate filament precursor	1	HUMIFP	
6.2-kDa protein	1	NM_019059	
Arg/Abl-interacting protein ArgBP2 (ARGBP2), transcript variant 1	1	NM003603	
Arg/Abl-interacting protein ArgBP2 (ARGBP2), transcript variant 2	1	NM021069	
ATPase, class VI, type 11B (ATP11B)	1	XM_087254	
CGI-56 protein	1	HSA011117	
Chemokine-like factor super family 8 (CKLFSF8)	1	XM_087402	
Coagulation factor C (limulus polyphemus) homology (Coch-5B2)	4	COCH	+
Cystein-rich protein (CROIP) gene, exon 5, 6	1	HUMCRP04	
deK (putative oncogen)	1	HS298J15	
DLEC1 to DRCTL4 gene region section 1/2	2	AB026898	
Down syndrome critical region protein A (DCAA)	1	NM006052	
Enhancer of rudimentary (<i>Drosophila</i>) homolog (ERH)	2	NM_004450	+
Finkel-Biskis-Reilly murin sarcoma virus (FBR-MuSV) ubiquitously expressed (fox derived)	2	NM006522	
Gene trap ankyrin repeat (GTAR)	1	AB014597	+
HIV-1 TAR binding protein	1	HUMTAP2	
HLA class III region containing NOTCH4 gene, homeobox	1	HSMHC3W5A	
Jun D proto-oncogene (JCND)	1	JUND	
KIAA0941 protein (= rab11-family interacting protein 2)	1	AB023158	
Kiss-1 metastasis-suppressor	5	NM002256	
Mu-crystallin gene, exon 8	1	HSMVCRY6	+
NY57-antigen	4	AF115514	
Org/ALI-interacting protein Arg BO2 & translated products (transcript varinat 2)	1	NM021069	
PAR (Parader-Willi/Angleman region) 5 gene from brain, 15q11-q13	1	AF019618	
Prohibin	1	PHB	
Rearranged mRNA for glutamine synthase	1	X59834	
Ring finger protein 11	1	AB024703	+
S-adenosyl homocysteine hydrolase-like	1	AHCYC1	
Selenium binding protein 1	2	NM_003944	

Table 2. Continued

Gene description	Occurrence	Accession no.	Identical to cochlear ESTs
Selenoprotein P, plasma 1 (SEPP1)	3	SEPP1	+
Serine (or cysteine) proteinase inhibitor, member 2	1	BC015663	
SET translation (myeloid leukemia-associated)	1	NM003011	
Similar to selenium binding protein 1	1	BC009084	
STX16 (syntaxin16)	1	HSJ907D15	
Testis-specific Y-encoded-like protein (TSPYL)	1	AF042181	
The HMG2L 1 gene for high molecultity group protein 2-like 1	1	HS510H16	
The N-O oct5a POU domain proteins	1	HS273N12	
Thyroglobulin gene, exon 32	1	HSTHRG08	
Thyroid autoantigen 70kD (ku antigen) (G22P1)	1	XM010020	
Transposon-derived Buster1 transposase-like protein	1	AF205600	
V-ski avian viral oncogene homolog	1	SKI	
Wbscr1 and replication factor C subunit 2	1	AF045555	
Unknown			
CGI-18 protein	1	AF151814	
cDNA DKFZp434H0115	1	HSM801728	
cDNA DKFZp434E2023	1	AL512761	
cDNA DKFZP566N1346 (= DKFZp762K0911)	2	AL110256	
cDNA DKFZp586P1622	1	HSM800861	
cDNA DKFZp686K0273	1	AL833622	
cDNA DKFZp686P072	1	AL832213	
cDNA DKFZp761F0621	1	AL713742	
cDNA FLJ 12116 (hypothetical protein)	1	AK022178	
cDNA FLJ 13794	2	AK023856	
cDNA FLJ 21778	1	AK025431	+
cDNA FLJ 37657	1	AK094976	
Chromosome 6 open reading frame 1	1	AY062936	
Chromosome 11 open reading frame 15	2	AJ400877	
Clone IMAGE:4042587, mRNA	1	BC021694	
Clone IMAGE:4052238, mRNA	1	BC014384	+
Clone IMAGE:4097766, mRNA	1	BC032596	
Clone IMAGE:5528612, mRNA	1	BC016735	
Clone IMAGE:5752707, mRNA	1	BC036789	
HepG 3' region cDNA	1	D16932	
Hypothetical protein CAB 56184	1	BC014592	
Hypothetical protein DKFZp564K0822	1	NM_030796	
Hypothetical protein FLJ21016	3	XM_015303	
Hypothetical protein FLJ21047	1	BC019890	
Hypothetical protein MGC 5350	1	XM_086221	
KIAA0100 gene product	1	XM_031460	
KIAA0537 gene product	2	AB011109	
KIAA0551 protein	3	AB011123	
KIAA0828 protein	1	XM_045904	
Nop 10p	1	AB043103	
Novel protein similar to predicted chicken protein	1	AL078581	
Similar to RIKEN cDNA 1110017I16 gene	2	BC018068	+

aspects in the fetal cochlea library database (<http://hearing.bwh.harvard.edu/cochlearcnlibrary.htm#ESTproduction>).

Semiquantitative RT-PCR and inner-ear expression

We performed RT-PCR experiments for the 22 selected EST clones of unknown function to confirm expression in the vestibule and eight other tissues. Nine of 22 ESTs were confirmed to be expressed in the vestibular tissue, as shown in Table 3. The expression of the two representative ESTs (clones 297 and 402) was confirmed in the inner ear (vestibule) and other organs (Fig. 1).

Discussion

We have reported here the successful construction of a human vestibular cDNA library by means of T7-based amplification of RNA. Although several methods were used previously for constructing mammalian inner-ear cDNA libraries, each library required a significant quantity of mRNA (Heller et al. 1998; Harter et al. 1999); for example, the well-established human fetal cochlear library required tissue from more than 100 fetal ears (Robertson et al. 1994; Skvorak et al. 1997). Because the collection of adequate amounts of inner ear tissue is so difficult, the number of clones obtained from a library will necessarily be limited.

Table 3. Expression profile analysis of nine novel ESTs by RT-PCR

Clone no.	Accession no.	Vestibule	Brain	Heart	Lung	Liver	Placenta	Kidney	Skeletal muscle	Pancreas
18	AB072818	+	—	+	+	+	—	+	+	—
35 ^a	AB072817	+	+	—	—	+	+	—	—	—
36 ^a	AB077323	+	+	—	—	+	+	—	—	—
124	AB072821	+	+	+	+	+	+	+	+	—
129	AB072822	+	+	—	+	+	+	—	—	+
229	AB072823	+	+	+	—	+	+	+	+	+
297	AB072825	+	—	—	—	—	—	—	—	—
360	AB074133	+	+	+	+	—	+	+	—	—
402	AB076958	+	+	—	—	—	+	—	—	+

ESTs, Expressed sequence tags; RT-PCR, reverse transcriptase-polymerase chain reaction; + detected; — not detected

^aNo. 35 and 36 ESTs share the same nucleotide sequences, and are considered to be derived from the same gene

Fig. 1. Expression of vestibular cDNA clones (No. 297 and 402), estimated by reverse transcriptase-polymerase chain reaction (RT-PCR). A 2.5% agarose gel stained with ethidium bromide shows RT-PCR products obtained from aRNAs extracted from various tissues. One cycle of T7-based RNA amplification was performed in total RNA from the vestibule and eight other organs. Human glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) serves as a positive control for the RNA

To overcome this primary disadvantage, we applied the T7-based RNA-amplification method (Van Gelder et al. 1993; Luo et al. 1999). Our results demonstrated that information about gene expression in the vestibule could be obtained from an extremely small amount of RNA. Although insert sizes of selected clones from our vestibular cDNA library were not as large as those of cochlear cDNA libraries reported previously, the almost complete nature of our genomic sequence information made it easy to define the transcription unit. With regard to repetitive elements, the insufficient sequence complexity, similar to that described in Morton's cochlear cDNA library, might have been enhanced to produce excess artifacts, and all repetitive elements were excluded in this study. The T7-amplification method was able to amplify the transcripts by maintaining the proportion of the original mRNA species, and the T7-amplified cDNA would be an almost complete representation of mRNA *in vivo*. Thus, identification of genes specifically expressed in the inner ear may be an effective approach to clarify which gene products play important

roles in the auditory and vestibular systems and also to discover and characterize genes that carry mutations responsible for deafness. Characterization of our vestibular cDNA library showed a wide variety of molecules including many unknown function proteins, suggesting that yet additional gene products may be involved in the vestibular function. RT-PCR analyses of the selected 22 EST sequences showed that 9 ESTs were expressed in the inner ear. However, no expression of the other 13 ESTs was detected in any tissues, and the possibility cannot be excluded that this problem may be due to an artifact such as unexpected genomic sequence. To evaluate whether these 9 EST transcripts would be truly novel gene fragments, we need further investigation.

In the human, a few cochlear cDNA libraries have been reported (Robertson et al. 1999; Jacob et al. 1997), but until now no vestibular cDNA library was available. When we compared results from our vestibular cDNA library with those of the fetal cochlear library, we noted that many genes of known functions were common in both libraries. How-

ever, dozens of genes detected in our vestibular library were not found in the fetal cochlear library, indicating that a specific set of genes is expressed in the vestibule.

Developmentally, the cochlea and vestibule originate from one embryonic organ, the otic capsule. The vestibular organ is remarkably similar to the cochlea, although their functions are very different. Some structures, including sensory hair cells, neuroepithelia, tectorial or otolithic membranes, and supporting cells, are common in both organs, suggesting that genes expressed in these two parts of the inner ear would play similar roles. Therefore, it is not surprising that the cDNA profile of our vestibular library significantly overlapped with that of the cochlear library. In fact, we encountered the *COCH* gene four times, the mutations of which are causative to nonsyndromic deafness with vestibular symptoms (DFNA9); this gene is reported to be highly expressed in the human cochlea and vestibule (Robertson et al. 1998). It is also of interest to know how vestibular function remains intact for many patients whose deafness is caused by mutations of nonsyndromic deafness genes expressed in both the vestibular and cochlear organs. Compared with the cochlear ESTs, particularly with respect to the phenotypic differences, the lack of vestibular dysfunction among affected patients who are carrying mutations in the deafness genes may reflect neurobiological differences based on gene expression profiling. The present study revealed only a small part of the human vestibular cDNA sequences. Nevertheless, the vestibular cDNA library that we have reported here represents a valuable experimental tool for investigating genes related to deafness and should contribute important insights into the molecular basis for the vestibular function.

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