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Gene expression dataset for whole cochlea of *Macaca fascicularis*

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***Macaca fascicularis* is a highly advantageous model in which to study human cochlea with regard to both evolutionary proximity and physiological similarity of the auditory system. To better understand the properties of primate cochlear function, we analyzed the genes predominantly expressed in *M. fascicularis* cochlea. We compared the cochlear transcripts obtained from an adult male *M. fascicularis* by macaque and human GeneChip microarrays with those in multiple macaque and human tissues or cells and identified 344 genes with expression levels more than 2-fold greater than in the other tissues. These "cochlear signature genes" included 35 genes responsible for syndromic or nonsyndromic hereditary hearing loss. Gene set enrichment analysis revealed groups of genes categorized as "ear development" and "ear morphogenesis" in the top 20 gene ontology categories in the macaque and human arrays, respectively. This dataset will facilitate both the study of genes that contribute to primate cochlear function and provide insight to discover novel genes associated with hereditary hearing loss that have yet to be established using animal models.**

Although the basic histological components of cochlear tissues are consistent among mammalian species[1], each species has a unique range of auditory frequencies[2] to perceive environmental change and communicate. This physiological variation can be explained not only by the morphological properties of conductive auditory organs such as the auditory canal, eardrum, and ear ossicles but also by the magnitude of expression of cochlea-specific genes. *Macaca fascicularis* (also called long-tailed, cynomolgus, or crab-eating macaque) is one of the best-studied nonhuman primate models for biomedical research; the entire genome has been sequenced, and most of the genes have been annotated[3,4]. The extremely high similarity between human transcripts and those of *M. fascicularis* as well as *Macaca mulatta* (rhesus macaque) has enabled investigators to study the gene expression profiles of macaque tissues using both the macaque and human microarray platforms[5]. The hearing range of *M. fascicularis* is from <0.1 to >32 kHz[6], which overlaps with human hearing rage from <0.1 to 20 kHz. Therefore, *M. fascicularis* is considered a highly advantageous model to study human cochlea with regard to both the evolutionary proximity and physiological similarity of the auditory system.

To date, more than 100 genes have been identified that are associated with nonsyndromic hearing loss in humans[7], and the number is increasing. While most of the genetic studies of cochlea have been carried out using rodents or other vertebrate species, the number of genetic studies using human cochlea is limited, mainly due to the difficulties in obtaining fresh cochlear tissues. Gene expression analysis from postmortem, formalin-fixed human cochlea is challenging due to fragmentation of the nucleic acids during fixation followed by decalcification and paraffin-embedding[8], therefore would provide limited information for biomedical research. In this study, we sought to generate the profile of genes predominantly expressed in freshly-dissected whole cochlear tissue of *M. fascicularis*, which should include genes critical to cochlear function.

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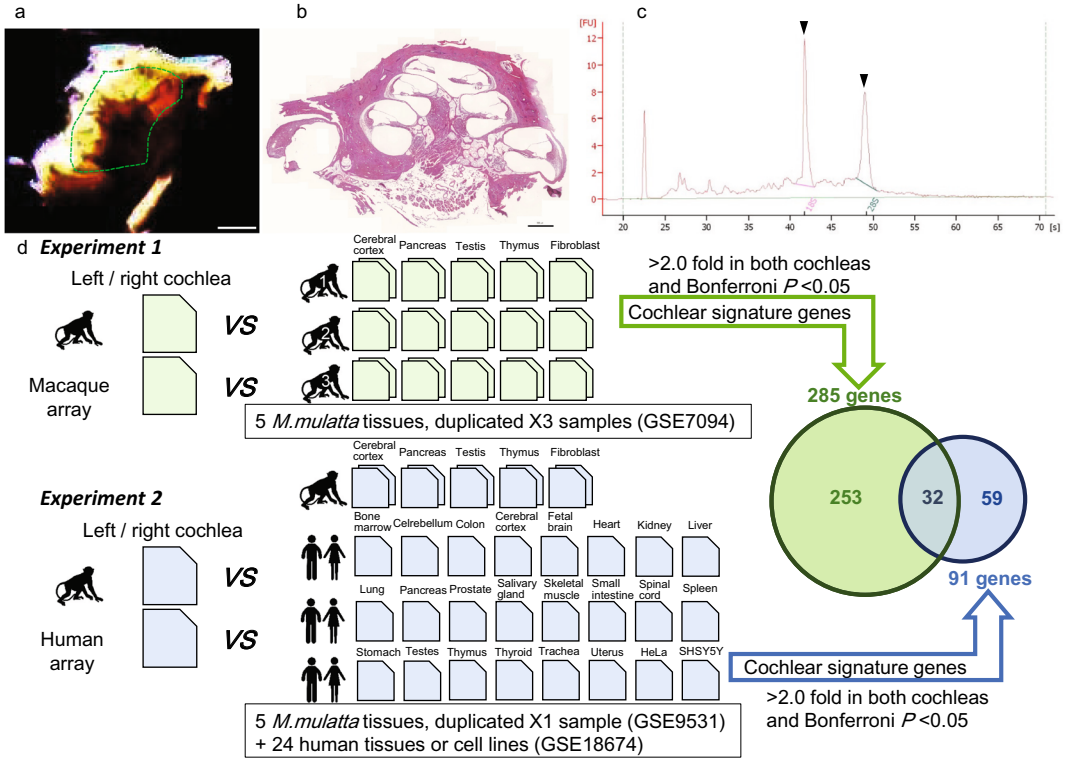


Figure 1. Schematic procedures to extract cochlear signature genes from *M. fascicularis*. (**a**) A dissected cochlea along with the modiolus. Tissues shown within the green dotted line were dissected out as whole cochlea and subjected to RNA extraction. Scale bar = 1 cm. (**b**) Histochemical image of a *M. fascicularis* cochlea stained with hematoxylin and eosin to show that the dissected "whole cochlea" in (**a**) corresponds to the membranous tissues of the cochlea. Scale bar = 500 μm. (**c**) Evaluation of the quality of RNA extracted from the left cochlea, as assessed with a Bioanalyzer 2100. Arrowheads indicate peaks of 18S and 28S rRNA. (**d**) Procedures of the analysis. Individual gene expression data in the left and right cochleae using the (experiment 1, top) macaque or (experiment 2, bottom) human microarray were compared with averaged expression levels of three or one macaque animals in duplicate and/or pooled human tissues or cells to extract probes that had expression levels >2-fold compared with the average of all the tissues and $P < 0.05$ (Welch's *t*-test with Bonferroni correction). Pentagons indicate array chips.

Results

Bilateral, whole cochlear tissues were freshly dissected from a male *M. fascicularis*, and total RNA was extracted immediately (Fig. 1a–c). We studied gene expression in the tissues and formulated a list of genes with expression levels >2-fold higher in the cochlea compared with (experiment 1) four tissues and a cell line from three independent *M. mulatta* animals[5] on Rhesus Macaque Genome Array (macaque array) with each biological sample in duplicate; or (experiment 2) four tissues and a cell line from one *M. mulatta* animal[5] and 22 pooled human tissues and 2 cell lines[9] on Human Genome U133 Plus 2.0 Array (human array, Fig. 1d). Since the transcripts between *M. mulatta* and *M. fascicularis* show almost 100% identity[3], the best platform to study gene expression in *M. fascicularis* cochlea would be macaque array. However, the platform was less frequently used (300 analyses have been registered in Gene Expression Omnibus (GEO), last visited on August 1, 2018) and predominantly for studies of viral infection or medical interventions in macaques. The number of available datasets of normal, untreated macaque tissues on the macaque array seemed limited to select genes predominantly expressed in the cochleae. The human array has been widely used (6,254 analyses in GEO, last visited on August 1, 2018) including multiple datasets of normal tissues, and was considered useful for meta-analysis to extract genes predominantly expressed in cochlear tissues. While affinities of the probes on the human array to the transcripts in the macaque tissues seemed not identical to those on macaque array[5], the transcripts between human and *M. fascicularis* show more than 95% identity[3], suggesting that profiles of gene expression in macaque cochleae can be analyzed on human array platforms in substitution.

Reproducibility of the datasets in the human microarray data was assessed by measuring Person's correlation coefficient and scatter plot analyses (see Supplementary Fig. S1). Small numbers of probes showing more than 2-fold changes, high values of correlation coefficient (>0.99) between tissue replicates, and the scatter plot analyses also indicated reproducibility of the datasets in each tissue. To verify the tissue-specific gene expression in macaque cochlea, 45,902 probes detected in at least one of the macaque cochleae on human array were subjected to cluster analysis among macaque cochleae and 22 human tissues (see Supplementary Fig. S2), demonstrating that related tissues such as those in central nervous system (cortex, cerebellum, fetal brain, spinal cord) were

clustered in the same group, and the macaque cochleae were closely related to the central nervous systems, suggesting that the datasets obtained in this study reflected actual profile of gene expression in the macaque cochlea.

Finally, we detected 474 probes that reflected the actual profile of 285 gene expression in experiment 1 and detected 99 probes that reflected the actual profile of 91 gene expression in experiment 2, and these genes were called cochlear signature genes (Table 1, see Supplementary Table S1). Of these genes, 32 were detected in both experiments 1 and 2, so the total number of cochlear signature genes was 344. The coincidence of the 32 genes was significant ($p < 2.2 \times 10^{-16}$, Fisher's exact test), verifying the reproducibility of experimens 1 and 2. The "common" cochlear signature genes and the expression profile among the examined tissues are shown as a heat map in Fig. 2. Intriguingly, the cochlear signature genes included 35 genes responsible for nonsyndromic or syndromic hearing loss such as *COCH* which is responsible for autosomal dominant nonsyndroic heaing loss (DFNA9, OMIM #601369)[10] and predominantly expressed in cochlear lateral wall, and *GJB2* which is responsible for autosomal recessive nonsyndromic hearing loss (DFNB1A, #220290)[11], the deafness gene most frequently found world wide (Table 2). Some of other examples were; *TYR* which is associated with ocular albinism and sensorineural deafness (#103470)[12], and *SLC17A8*, a marker gene for spiral ganglion cells in the cochlea and responsible for autosomal dominant nonsyndromic deafness (DFNA25, #605583)[13].

Gene ontology analysis of the datasets identified 434 enriched terms in the macaque array and 685 enriched terms with $P < 0.05$. As expected, groups of genes categorized to "ear development" and "ear morphogenesis" were included in the list of top 20 gene ontology categories in the macaque and human arrays, respectively (see Supplementary Table S2 and Supplementary Fig. S3).

Among the common cochlear signature genes, we attempted to compare expression levels of *COCH*, *IL17B*, and *NEK1* in the macaque cochleae with those in a human brain by quantitative RT-PCR (qRT-PCR, see Supplementary Fig. S4). Comparison of gene expression/*GAPDH* ratios indicated expression of the all three genes in the macaque cochleae was significantly higher than the human brain, partially reproducing the predominant expression of cochlear signature genes in the macaque cochlea.

Discussion

Our study presents the profile of cochlear signature genes obtained from bilateral whole cochleae dissected from an adult male *M. fascicularis*. Based on the facts that 1) datasets detected in the macaque cochleae were suggested to reflect actual profile of gene expression by cluster analysis; 2) cochlear signature genes were enriched in genes associated with nonsyndromic or syndromic hearing loss in both microarray platforms; 3) genes categorized to ear development or ear morphogenesis were highly enriched by gene set enrichment analysis in both microarray platforms, we coclude that the method to extract cochlear signature genes using the two microarray platforms was valid.

There have been transcriptomic analyses of sensory hair cells and the progenitor cells in zebrafish lateral line[14], regenerating chicken utricle hair cells after ototoxic drug treatment[15], embryonic to newborn mouse inner ear sensory cells[16] or ganglion cells[17], or proteomic analysis of newborn mouse inner ear hair cells[18], all of which have focused mainly on differentiation and/or regeneration of inner ear sensory hair cells or neurons. Cell type-specific analysis results in paying less attention to the surrounding non-sensory cochlear tissues, which play significant roles in normal cochlear function. Using whole cochlear tissues, we have successfully detected cochlear signature genes including *MLANA*[19] as well as *TYR*[12], both of which are marker genes for the melanocyte (also called as intermediate cell) in the stria vascularis. *COCH*[10] and *GJB2*[11], both of which are responsible for hereditary hearing loss and are expressed predominantly in the cochlear tissues other than organ of Corti were also included in the gene list, supporting the anticipation that the genes with significant roles in the cochlea show predominant expression levels in the tissues. More than 10% of the cochlear signature genes (35 out of 344) was estimated to associate with hereditary syndromic or nonsyndromic hearing loss. Since several hundreds, but not thousands of genes have been roughly predicted to associate with hereditary hearing loss[20–22] in all the human genes (approximately 19,000–20,000)[23,24], cochlear signature genes are presumably rich in deafness genes. Regarding the fact that novel genes associated with hearing loss have been reported every year, it raises the possibility that unreported deafness genes are included in the cochlear signature genes. One possible application of the cochlear signature genes would be to use the list to prioritize the candidate deafness genes from the results of whole exome/genome sequencing when there are no other evidence of clinical data or animal experiments associated with hearing loss.

Limitation of this study is that the gene expression data was based on bilateral cochleae from one animal, and it was not possible to conduct the statistical analysis among multiple animals to show the variance among animals. During tissue dissection, we found it extremely challenging to obtain high quality total RNA from whole cochlear tissues surrounded by thick temporal bones in macaque. During our limited opportunities to optimize how to extract RNA from several euthanized macaques, we found that only the fresh cochlear tissues dissected within 30 minutes after sacrifice and before formalin perfusion enabled recovery of total RNA with high quality (that is, RIN $\geq$ 7.0) for microarray analysis. The datasets presented here were obtained to minimize degeneration of RNA in the macaque cochleae and therefore valuable, even if the data came from bilateral cochleae from one individual animal. Increasing the number of macaques for the examination will enable the statistical analysis in the future and, perhaps, decrease the number of cochlear signature genes. The reason that cochlear signature genes extracted from the macaque array outnumbered those of the human array was considered to reflect the fact that a limited number of datasets was used for comparison in the case of the macaque array.

The profile of cochlear signature genes obtained from high-quality RNA, two array GeneChip platforms (including the widely used human array), and extensive comparison with five macaque tissues and 24 human tissues or cell lines constitutes a valuable resource for studies of genes that contribute to cochlear structure and function in primates, and provide insight to discover novel genes associated with hearing loss that have yet to be established in rodent models.

Macaque array chip			Human array chip
MLANA	*EBF2*	*FRMD3*	*KRTDAP*
COCH	*GFRA1*	***IL17B***	***COCH***
PSMD12	*SULF1*	*ANXA3*	*EPYC*
SLCO2B1	*WDR86*	*ARL9*	***OTOS***
MPZ	*MEPE*	*TMPRSS11E*	***NEFH***
S100B	*SFRP4*	*PROM1*	***DNASE1***
OTOS	*SLC4A11*	*PLEKHG7*	*FLG2*
SMPX	*LOC693624*	*RPL24*	***SLC17A8***
COL10A1	*ANXA4*	*DMKN*	***MLANA***
TUFM	***KIAA1024***	*UACA*	*SERPINB6*
GJB6	*TNNT1*	*EBF3*	*SLC17A6*
NEFH	*KIF21A*	*FGFR2*	*NDP*
ANO5	*OSMR*	*CPXM2*	*KCTD4*
MLIP	*MS4A6A*	*COLEC12*	*C17orf67*
LOR	*UBA6*	*ABLIM1*	***MPZ***
SHC4	*CRABP1*	*B3GNT5*	*NRG1*
SLC22A2	*BMP6*	*GSN*	***COL10A1***
PVALB	*SIGLEC9*	*CDK2*	*OTOR*
ZIC2	*MAB21L1*	*LXN*	*cDNA FLJ43186 fis*
UGT8	*RTFDC1*	*MEGF10*	***KCNJ13***
SCEL	*PPARGC1A*	*SLC26A4*	*LANCL3*
LRP2	*HKDC1*	*MRPS26*	***TYR***
LOC718942	*HTRA1*	*LASS3*	*PLEKHA4*
SLC27A6	*CRISP3*	*CYP26A1*	*LOC100288310*
SLCO1A2	*DUT*	*NIPSNAP3B*	***DEFB122***
TYR	*LTBP4*	*CYB5R3*	*cDNA IMAGE:1625225*
SLC17A8	*KRT23*	*VASH2*	***OGN***
KRT24	*ALDH1A3*	*S100A10*	*GJB2*
SCIN	*SPTLC3*	*OAS1*	*CALCA*
SV2C	*RDH10*	*IRX6*	*LECT1*
GAS2	*TM4SF18*	*IL18*	***LOR***
SPP1	*SMCO3*	*HPGD*	*PCP4*
DMP1	*CTXN3*	*SLCO1B1*	*EYA1*
DCLK3	*SCN7A*	*HOXD1*	***SHC4***
Mamu_482871	*GPR87*	*MORF4L1*	***KCNB2***
PTN	*ITGB8*	***RBMS3***	*IRX5*
PheRS	*CRTAC1*	*DACH1*	***PTN***
SERPIND1	*UPK1B*	*LOC696306*	***IL17B***
CLDN8	*MRAP2*	*MFAP3L*	***KIAA1024***
TNFRSF11B	*WDR18*	*FIBIN*	*POU4F2*
DSC2	*FIG4*	*GRHL1*	***CLCA2***
VTCN1	*STAC*	*TFAP2B*	*POU4F1*
C19H19orf33	*SCARA5*	*CPSF6*	*PLCB4*
LOC717747	*TNFRSF19*	*LOC720403*	*cDNA FLJ37676 fis*
CRYAB	*MGP*	*PIK3R1*	***EBF1***
LGR5	*BCAS1*	*GAL3ST1*	*C12orf69*
OVOS	*CLIC5*	*WDR11*	*FLG*
IBSP	*OLFM4*	*SCUBE2*	*KCNN2*
CP	*CCDC114*	*FREM1*	*LRRN1*
PAPSS2	*SVIP*	*C1ORF162*	*KCNQ4*
P2RY2	*MS4A7*	*GANC*	***AADACL2***
KLK7	*F13A1*	*UST*	*MAGI1*
GJB4	***CST6***	*ACSL1*	***KRT24***
ESRRG	*INSC*	*GDPD3*	*CARD18*
CLCA2	*CDH19*	*WNK3*	***ESRRG***
OGN	*RERGL*	*ERMP1*	*RAB12*
DEFB122	*PAX3*	*GPC6*	***SLC13A4***

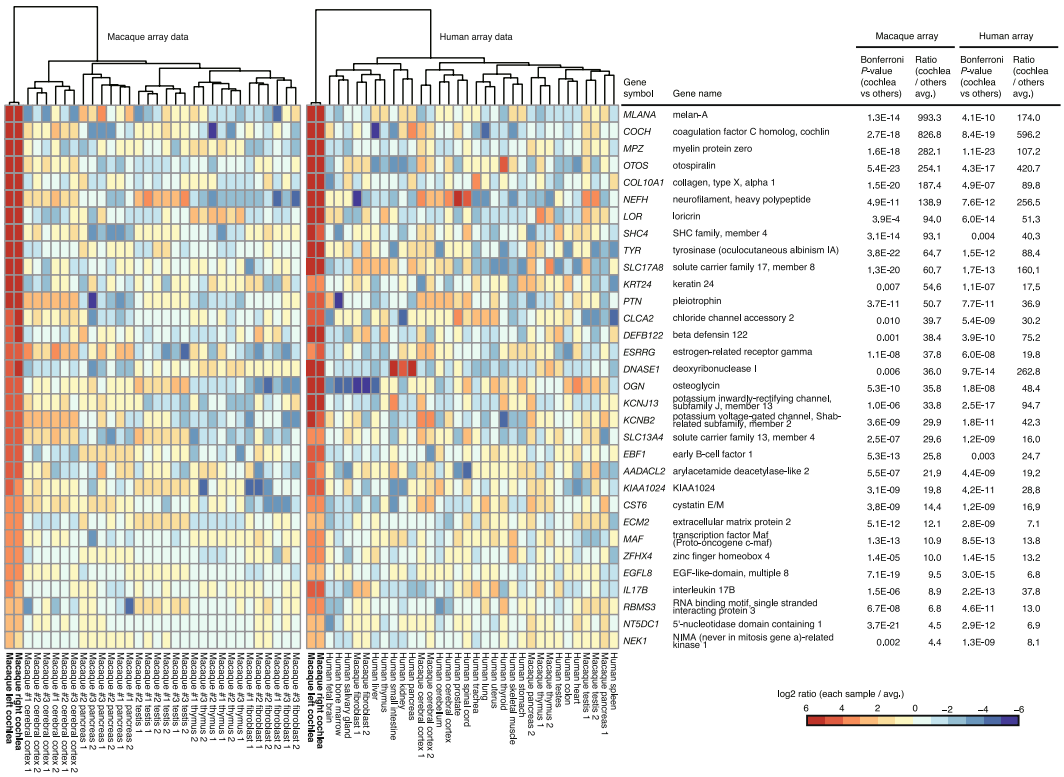
Continued

Macaque array chip			Human array chip
OMD	*HEY2*	*CSRP2*	***CST6***
SLC13A4	*ELOVL7*	*IFIT1*	*cDNA DKFZp686P21116*
DNASE1	*MALL*	*MPP6*	*LOC283143*
KCNB2	*PPP1R1C*	*BGLAP*	*FIGN*
OTOGL	*PROS1*	*MPZL2*	*FAM190A*
SSBP1	*HHATL*	*MTMR6*	*RAD54B*
KCNJ13	*CALML5*	*EEF1D*	*GRIK2*
MAPK8IP2	*LOC693471*	*NPNT*	***RBMS3***
DSC1	*LDLRAD3*	*FAM162A*	***MAF***
EYA4	*EGR2*	*CD55*	***ZFHX4***
FMO3	*PTPRU*	*GTPBP8*	*LOC220077*
PMP22	*LOC694405*	*RORA*	*DDR2*
TRPM1	*EFEMP1*	*TMCC3*	*NFIB*
TMEM213	*IGHV4OR15-8*	***NEK1***	*AKAP12*
ABCA10	*PRX*	***NT5DC1***	*ITGA10*
RDH5	*OLR1*	*GSTM4*	*CADM1*
PLLP	*SERTAD4*	*DLGAP5*	*TMEM117*
CA14	*SPAG11*	*PXK*	*RARB*
CA13	***ECM2***	*CCL26*	*PLAG1*
COL4A4	*STX1B*	*PBX3*	*SECISBP2L*
METTL7B	*EIF5A2*	*COL11A2*	*LPIN1*
ITIH2	*SLC25A13*	*C15H9orf3*	***NEK1***
PRH2	*KCNE1*	*APBB2*	*ANK3*
FILIP1	***MAF***	*SCCPDH*	***ECM2***
RARRES1	*WNT16*	*EPM2A*	*SESN3*
FOXC1	*ZFHX3*	*TAB2*	***NT5DC1***
SOST	*OR51E2*	*FNTA*	*CCDC126*
EBF1	*ID4*	*SBF2*	***EGFL8***
COL8A2	*SLC5A3*		*cDNA IMAGE:3565734*
C2ORF40	*PFKFB3*		*ITSN1*
ASPA	*SOX17*		*USP32*
CES1	*CFB*		*DST*
CHST9	*WFDC5*		*PSMA2*
COL2A1	*ABI3BP*		*C15orf40*
AADACL2	*MCOLN3*		
ABCA9	*LOC702904*		
MUC15	*GPR137B*		
CRISPLD1	*SPTBN1*		
IPO5	*TM7SF2*		
SPATA22	*MEOX2*		
IL20RB	*COL9A3*		
CTDSP2	***EGFL8***		
DCP2	***ZFHX4***		

Table 1. List of Cochlear signature genes detected on macaque or human array chip platform. Gene symbol in bold indicates that the gene is found on both macaque and human array chip platforms.

Methods

Animal care. The test animal was a 5-year-old male Malaysian *M. fascicularis* housed at the Tsukuba Primate Research Center (TPRC), National Institutes of Biomedical Innovation, Health and Nutrition (NIBIOHN), Tsukuba, Ibaraki, Japan. The animal was cared for, handled, and sacrificed according to the guidelines and regulations established by the Institutional Animal Care and Use Committee of NIBIOHN and the standard operating procedures for macaques at TPRC. The animal was housed individually in a size-appropriate cage, and the light cycle consisted of 12 h of artificial light from 7 am to 7 pm. Temperature and humidity were maintained at 25 ± 2 °C and 60 ± 10% in the animal room. The animal was fed 70 g of commercial monkey chow (Type AS; Oriental Yeast Co., Ltd., Tokyo, Japan) and 100 g of apples daily. Water was supplied *ad libitum*. All experimental procedures were approved by the Institutional Animal Care and Use Committee of NIBIOHN. Although the macaque subjected for this study had not been examined by auditory brainstem response nor by otoacoustic emissions, the animal had never shown any behaviors suspicious for hearing impairment while kept in the facility, such as ignorance to the sound. The animal was kept in the room with the environmental noise kept to below



Gene symbol	Gene name	Macaque array: Bonferroni *P*-value (cochlea vs others)	Macaque array: Ratio (cochlea / others avg.)	Human array: Bonferroni *P*-value (cochlea vs others)	Human array: Ratio (cochlea / others avg.)
MLANA	melan-A	1.3E-14	993.3	4.1E-10	174.0
COCH	coagulation factor C homolog, cochlin	2.7E-18	826.8	8.4E-19	596.2
MPZ	myelin protein zero	1.6E-18	282.1	1.1E-23	107.2
OTOS	otospiralin	5.4E-23	254.1	4.3E-17	420.7
COL10A1	collagen, type X, alpha 1	1.5E-20	187.4	4.9E-07	89.8
NEFH	neurofilament, heavy polypeptide	4.9E-11	138.9	7.6E-12	256.5
LOR	loricrin	3.9E-4	94.0	6.0E-14	51.3
SHC4	SHC family, member 4	3.1E-14	93.1	0.004	40.3
TYR	tyrosinase (oculocutaneous albinism IA)	3.8E-22	64.7	1.5E-12	88.4
SLC17A8	solute carrier family 17, member 8	1.3E-20	60.7	1.7E-13	160.1
KRT24	keratin 24	0.007	54.6	1.1E-07	17.5
PTN	pleiotrophin	3.7E-11	50.7	7.7E-11	36.9
CLCA2	chloride channel accessory 2	0.010	39.7	5.4E-09	30.2
DEFB122	beta defensin 122	0.001	38.4	3.9E-10	75.2
ESRRG	estrogen-related receptor gamma	1.1E-08	37.8	6.0E-08	19.8
DNASE1	deoxyribonuclease I	0.006	36.0	9.7E-14	262.8
OGN	osteoglycin	5.3E-10	35.8	1.8E-08	48.4
KCNJ13	potassium inwardly-rectifying channel, subfamily J, member 13	1.0E-06	33.8	2.5E-17	94.7
KCNB2	potassium voltage-gated channel, Shab-related subfamily, member 2	3.6E-09	29.9	1.8E-11	42.3
SLC13A4	solute carrier family 13, member 4	2.5E-07	29.6	1.2E-09	16.0
EBF1	early B-cell factor 1	5.3E-13	25.8	0.003	24.7
AADACL2	arylacetamide deacetylase-like 2	5.5E-07	21.9	4.4E-09	19.2
KIAA1024	KIAA1024	3.1E-09	19.8	4.2E-11	28.8
CST6	cystatin E/M	3.8E-09	14.4	1.2E-09	16.9
ECM2	extracellular matrix protein 2	5.1E-12	12.1	2.8E-09	7.1
MAF	transcription factor Maf (Proto-oncogene c-maf)	1.3E-13	10.9	8.5E-13	13.8
ZFHX4	zinc finger homeobox 4	1.4E-05	10.0	1.4E-15	13.2
EGFL8	EGF-like-domain, multiple 8	7.1E-19	9.5	3.0E-15	6.8
IL17B	interleukin 17B	1.5E-06	8.9	2.2E-13	37.8
RBMS3	RNA binding motif, single stranded interacting protein 3	6.7E-08	6.8	4.6E-11	13.0
NT5DC1	5'-nucleotidase domain containing 1	3.7E-21	4.5	2.9E-12	6.9
NEK1	NIMA (never in mitosis gene a)-related kinase 1	0.002	4.4	1.3E-09	8.1

Figure 2. The 32 cochlear signature genes that were common to both the macaque array and the human array. Their expression levels are shown as a heat map that includes genes that clustered together in each of the macaque and human tissues. Gene symbols, gene names, Bonferroni-corrected *P*-values, and expression ratios with other tissues are shown on the right side of the map. "1" or "2" in each tissue or cell line indicates replication number.

60 dB. The animal did not have history of obesity, treatment with ototoxic drugs, nor exposure to loud sound, all of which are risk factors of age-related hearing loss[25]. Therefore, we considered that the animal had normal hearing at the time of experiment.

Tissue collection and RNA extraction. For RNA extraction, bilateral cochleae were dissected from the test animal within 30 minutes after sacrifice by exsanguination under deep anesthesia (Fig. 1a). First, the bony labyrinths were dissected from left and right temporal bone, then connective tissues were removed and placed in ice-cold saline. RNA from whole membranous cochlear tissues was extracted using ISOGEN-II (Nippon Gene, Toyama, Japan) and purified using an RNeasy micro kit (QIAGEN, Hamburg, Germany). Quality of the RNA extracted from the cochleae was analyzed with a Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA) (Fig. 1c).

Transcription profiling. Biotinylated antisense RNA (aRNA) from 250 ng total RNA was prepared from left or right cochlea separately according to the manufacturer protocols (Affymetrix, Santa Clara, CA, USA). Then, 10 μg of aRNA was hybridized on the GeneChip Rhesus Macaque Genome Array (macaque array, Affymetrix) and the Human Genome U133 Plus 2.0 Array (human array, Affymetrix) for 16 h at 45 °C (Fig. 1d). The GeneChip microarrays were washed and stained in the Affymetrix Fluidics Station 450. The stained GeneChips were scanned using the Affymetrix Scanner 3000-7G. The images were digitized using GeneChip Operating Software (GCOS) v1.3 (Affymetrix), and the data were exported as CEL files. The microarray data were normalized using the MAS5 algorithm (Affymetrix). The intensities were converted to a logarithmic scale (base 2). To correct for bias between arrays, we then performed quantile normalization for all array data using R software ("affy" and "limma" packages). The signal reliability of each probe was determined using the MAS5 Call algorithm (Affymetrix), and each probe was assigned to one of three flags: P, present; M, marginal; A, absent (GEO #GSE111693).

In addition, the pair of gene expression data in the left and right cochleae using the macaque microarray were compared with averaged expression levels of those in four tissues and a cell line (cerebral cortex, pancreas, testis, thymus, and fibroblast, three samples with duplicated data in each tissue or cell) of *M. mulatta* using the same platform (Fig. 1c, top) (GSE7094)[5].

The pair of gene expression data in the left and right cochleae using the human microarray were compared with averaged expression levels of those in the five tissues (one sample with duplicated data in each tissue or cell line) of *M. mulatta* (GSE9531)[5] in addition to 24 human tissues or cell lines (bone marrow, cerebellum, colon, cortex, fetal brain, heart, kidney, liver, lung, pancreas, prostate, salivary gland, skeletal muscle, small intestine,

Gene symbol	Disease	OMIM phenotype ID
Human and Macaque array		
COCH	Autosomal dominant deafness 9	601369
MPZ	Charcot-Marie-Tooth disease DID, type 1B, 2J	607791, 118200, 607736
LOR	Vohwinkel syndrome, variant form	604117
TYR	Albinism, ocular, with sensorineural deafness	103470
SLC17A8	Autosomal dominant deafness 25	605583
MAF	Ayme-Gripp syndrome	601088
Macaque array		
PSMD12	Stankiewicz-Isidor syndrome	617516
SMPX	Deafness, X-linked 4	300066
GJB6	Autosomal dominant deafness 3B, 1B	612643, 612645
LRP2	Donnai-Barrow syndrome	222448
TNFRSF11B	Paget disease of bone 5, juvenile-onset	239000
OTOGL	Autosomal recessive deafness 84A, 84B	613391, 614944
EYA4	Autosomal dominant deafness 10	601316
PMP22	Charcot-Marie-Tooth disease1A, 1E	118200, 118300
COL4A4	Autosomal recessive Alport syndrome	203780
FOXC1	Axenfeld-Rieger syndrome type 3	602482
SOST	Autosomal dominant Craniodiephyseal dysplasia, Van Buchem disease	122860, 239100
COL2A1	Stickler syndrome, type 1	108300
SLC4A11	Corneal dystrophy and perceptive deafness	217400
MGP	Keutel syndrome	245150
CLIC5	Autosomal recessive deafness 103	616042
PAX3	Waardenburg syndrome, type 1, 3	193500, 148820
EGR2	Congenital hypomyelinating neuropathy, Dejerine-Sottas disease	605253, 145900
PRX	Dejerine-Sottas disease	145900
KCNE1	Jarvell and Lange-Nielsen syndrome 2	612347
FGFR2	Crouzon syndrome, Pfeiffer syndrome, Apert syndrome, Antley-Bixler syndrome	123500, 101600, 101200, 207410
SLC26A4	Autosomal recessive deafness 4 with enlarged vestibular aqueduct, Pendred syndrome	600791, 274600
PIK3R1	SHORT syndrome, Immunodeficiency 36	269880, 616005
SBF2	Charcot-Marie-Tooth disease 4B2	604563
COL11A2	Autosomal dominant deafness 13, Autosomal recessive deafness 53, otospondylomegaepiphyseal dysplasia	601868, 609706, 184840
Human array		
SERPINB6	Autosomal recessive deafness 91	613453
NDP	Norrie disease	310600
GJB2	Audtosomal dominant deafness 3A, Autosomal recessive deafness 1A, Keratitis-ichthyosis-deafness syndrome	601544, 220290, 148210
EYA1	Branchiootorenal syndrome 1, Branchiootic syndrome 1	113650, 602588
KCNQ4	Autosomal dominant deafness 2A	600101

Table 2. List of genes associated with nonsyndromic or syndromic hearing loss detected in this study.

spinal cord, spleen, stomach, testes, thymus, thyroid, trachea, uterus, HeLa, and SHSY5Y, single data in each tissue) (Fig. 1c, bottom) (GSE18674)[9] and using the same platform. As for human tissues, total RNA of each tissue had been purchased from several providers and pooled from more than 10 individuals on average to minimize individual variations[9]. Cluser analysis was performed by Ward's method using R.

To identify cochlear signature genes, statistical significance was assessed with Welch's *t*-test with Bonferroni correction. Probes were extracted that had expression levels >2-fold compared with the average of all the tissues and corrected $P < 0.05$. Gene symbols were updated manually. Gene ontology analysis was conducted according to the Gene Set Enrichment Analysis software[26,27].

Gene expression levels measurement by qRT-PCR. Total RNA extracted from human brain (purchased from TaKaRa BIO, Shiga, Japan) or from the whole left or right cochlear tissues from the macaque was reverse transcribed by SuperScript III (ThermoFisher Scientific, Massachusetts, USA) and was subjected to qRT-PCR using PowerSybrGreen PCR Master Mix (Applied Biosystems, California, USA) and QuantStudio 3 (Applied Biosystems) according to the manufacture's protocols. Primer sets used in this study were shown in Supplementary Table S3. The experiment was evaluated as Gene expression/endogenously expressed *GAPDH* ratio with triplicate analyses of each experiment. Statistical evaluation was done by 2-way ANOVA.

Data Availability

The data described here can be found at Mutai, H. *et al*. GEO #GSE111693 (2018).

References

1. Ekdale, E. G. Comparative anatomy of the bony labyrinth (inner ear) of placental mammals. *PloS One.* **8**, e66624 (2013).
2. Long, G. R. Psychoacoustics in *Comparative Hearing:Mammals. Springer Handbook of Auditory Research* (eds Fay, R. R. & Popper, A. N.) Ch. 2, 18–56 (Springer-Verlag, 1994).
3. Ebeling, M. *et al*. Genome-based analysis of the nonhuman primate Macaca fascicularis as a model for drug safety assessment. *Genome Res.* **21**, 1746–1756 (2011).
4. Higashino, A. S. *et al*. Whole-genome sequencing and analysis of the Malaysian cynomolgus macaque (Macaca fascicularis) genome. *Genome Biol.* **13**, R58 (2012).
5. Duan, F., Spindel, E. R., Li, Y. H. & Norgren, R. B. Jr. Intercenter reliability and validity of the rhesus macaque GeneChip. *BMC genom.* **8**, 61 (2007).
6. Coleman, M. N. & Ross, C. F. Primate auditory diversity and its influence on hearing performance. *Anat. Rec. Part A.* **281**, 1123–1137 (2004).
7. Van Camp, G. & Smith, R. Hereditary Hearing Loss Home Page. http://hereditaryhearingloss.org (2018).
8. Kimura, Y. *et al*. RNA analysis of inner ear cells from formalin fixed paraffin embedded (FFPE) archival human temporal bone section using laser microdissection – A technical report. *Hear. Res.* **302**, 26–31 (2013).
9. Tateno, C. *et al*. Morphological and microarray analyses of human hepatocytes from xenogeneic host livers. *Lab. Invest.* **93**, 54–71 (2013).
10. Robertson, N. G. *et al*. Cochlin immunostaining of inner ear pathologic deposits and proteomic analysis in DFNA9 deafness and vestibular dysfunction. *Hum. Mol. Genet.* **15**, 1071–1085 (2006).
11. Kelsell, D. P. *et al*. Connexin 26 mutations in hereditary non-synbdromic sensorineural deafness. *Nature* **387**, 80–83 (1997).
12. Filimon, A. *et al*. Value of dopachrome tautomerase detection in the assessment of melanocytic tumors. *Melanoma Res.* **24**, 219–236 (2014).
13. Ruel, J. *et al*. Impairment of SLC17A8 encoding vesicular glutamate transporter-3, VGLUT3, underlies nonsyndromic deafness DFNA25 and inner hair cell dysfunction in null mice. *Am. J. Hum. Genet.* **83**, 278–292 (2008).
14. Steiner, A. B., Kim, T., Cabot, V. & Hudspeth, A. J. Dynamic gene expression by putative hair-cell progenitors during regeneration in the zebrafish lateral line. *Proc. Nat. Acad. Sci. USA* **111**, E1393–1401 (2014).
15. Ku, Y. C. *et al*. The transcriptome of utricle hair cell regeneration in the avian inner ear. *J. Neurosci.* **34**, 3523–3535 (2014).
16. Scheffer, D. I., Shen, J., Corey, D. P. & Chen, Z. Y. Gene Expression by Mouse Inner Ear Hair Cells during Development. *J. Neurosci.* **35**, 6366–6380 (2015).
17. Lu, C. C., Apller, J. M., Houseman, E. A. & Goodrich, L. V. Developmental profiling of spiral ganglion neurons reveals insights into auditory circuit assembly. *J. Neurosci.* **31**, 10903–10918 (2011).
18. Hickox, A. E. Global analysis of protein expression of inner ear hair cells. *J. Neurosci.* **37**, 1320–1339 (2017).
19. Locher, H. *et al*. Development of the stria vascularis and potassium regulation in the human fetal cochlea: Insights into hereditary sensorineural hearing loss. *Dev. Neurobiol.* **75**, 1219–1240 (2015).
20. Morton, C. C. & Nance, W. E. Newborn hearing screening- a silent revolution. *N. Engl. J. Med.* **354**, 2151–2164 (2006).
21. Brownstein, Z. *et al*. Targeted genomic capture and massively parallel sequencing to identify genes for hereditary hearing loss in Middle Eastern families. *Genome Biol.* **12**, R89 (2011).
22. Linden Phillips, L. *et al*. The future role of genetic screening to detect newborns at risk of childhood-onset hearing loss. *Int. J. Audiol.* **52**, 124–133 (2013).
23. Pennisi, E. ENCODE project writes eulogy for junk DNA. *Science* **337**, 1159 (2012).
24. Ezkurdia, I. *et al*. Multiple evidence strands suggest that there may be as few as 19000 human protein-coding genes. *Hum. Mol.Genet.* **23**, 5866–5878 (2014).
25. Fransen, E. *et al*. Occupational noise, smoking, and a high body mass index are risk factors for age-related hearing impairment and moderate alcohol consumption is protective: a European population-based multicenter study. *J Assoc. Res. Otolaryngol.* **9**, 264–276 (2008).
26. Mootha, V. K. *et al*. PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nat. Genet.* **34**, 267–273 (2003).
27. Subramanian, A. *et al*. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc. Nat. Acad. Sci. USA* **102**, 15545–15550 (2005).

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Author Contributions

H.M. designed the study, dissected cochlear tissues and prepared RNA, participated in analyzing data, conducted qRT-PCR, drafted and finalized the manuscript. F.M. participated in designing the experiment, conducted microarray analysis, and drafted the manuscript. H.S. and Y.Y. administered animal health, euthanized the animal, dissected the temporal bones, and drafted the manuscript. T.T. and T.M. conceived the study and contributed to the interpretation of the data.

Additional Information

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