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A comprehensive catalogue of the coding and non-coding transcripts of the human inner ear

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ABSTRACT

The mammalian inner ear consists of the cochlea and the vestibular labyrinth (utricle, saccule, and semicircular canals), which participate in both hearing and balance. Proper development and life-long function of these structures involves a highly complex coordinated system of spatial and temporal gene expression. The characterization of the inner ear transcriptome is likely important for the functional study of auditory and vestibular components, yet, primarily due to tissue unavailability, detailed expression catalogues of the human inner ear remain largely incomplete.

We report here, for the first time, comprehensive transcriptome characterization of the adult human cochlea, ampulla, saccule and utricle of the vestibule obtained from patients without hearing abnormalities. Using RNA-Seq, we measured the expression of >50,000 predicted genes corresponding to approximately 200,000 transcripts, in the adult inner ear and compared it to 32 other human tissues. First, we identified genes preferentially expressed in the inner ear, and unique either to the vestibule or cochlea. Next, we examined expression levels of specific groups of potentially interesting RNAs, such as genes implicated in hearing loss, long non-coding RNAs, pseudogenes and transcripts subject to nonsense mediated decay (NMD). We uncover the spatial specificity of expression of these RNAs in the hearing/balance system, and reveal evidence of tissue specific NMD. Lastly, we investigated the non-syndromic deafness loci to which no gene has been mapped, and narrow the list of potential candidates for each locus.

These data represent the first high-resolution transcriptome catalogue of the adult human inner ear. A comprehensive identification of coding and non-coding RNAs in the inner ear will enable pathways of auditory and vestibular function to be further defined in the study of hearing and balance. Expression data are freely accessible at https://www.tgen.org/home/research/research-divisions/neurogenomics/supplementary-data/inner-ear-transcriptome.aspx.

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1. Introduction

The inner ear consists of auditory and vestibular components that involve a highly complex coordinated system of spatial and temporal gene expression to control the connectivity of neurons and circuits. The vestibular components are further divided into the utricle, saccule and three semicircular ducts each with a terminal enlargement called an osseous ampulla. The large number of

List of abbreviations: NMD, nonsense mediated decay; lincRNAs, long intervening non-coding RNAs; FPKM, Fragments per kilobase of exon per million reads mapped; NATs, Natural antisense transcripts; ncRNAs, non-coding RNAs; T-UCRs, transcribed ultraconserved regions; AD, Autosomal dominant; AR, Autosomal recessive

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genetic mutations and loci that have been implicated in hearing and balance disorders further illustrate structural and functional complexity of this organ. Yet, much of what is known about the genetics of hearing in humans comes from the study of Mendelian disorders. More than 100 loci were identified in familial studies of nonsyndromic hearing loss, with ~30% of the studies failing to identify the specific gene or mutation implicated (http://hereditaryhearingloss.org/). The genetic basis for the more common and complex forms of hearing and balance disorders, such as presbycusis and disequilibrium and falls in the elderly, has been established (Fransen et al., 2014) but remains poorly understood.

Over the past decade, microarray technology has proven to be a useful tool for performing high throughput gene expression analyses of the inner ear. While initially used primarily to characterize the level of expression of mRNAs in the wild type tissue, it was later used to identify complex gene networks, decipher regulatory pathways and characterize response to various stimuli. Numerous studies have profiled the inner ear gene expression using microarray technology, focusing on model organisms such as mouse and zebrafish, both in normal and pathological conditions (Hertzano and Elkon, 2012; Scheffer et al., 2015). In contrast to the wealth of data available for model organisms, very little is known about gene expression in the human inner ear, mainly due to difficulties in obtaining the relevant tissues. In fact the only available genome wide profiling of the transcriptome in human inner ear was published in 1999 and 2002 by the laboratory of Dr. Cynthia Morton, using expressed sequence tags in human fetal cochlear tissue (Resendes et al., 2002; Skvorak et al., 1999). We believe that knowledge of the elementary expression patterns of the hearing/balance system in the functioning human ear will be a valuable tool for gaining systems-level understanding of basic biological processes during normal development, or development of pathological conditions and will allow significant progress in identification of new genes functionally important for human hearing.

Recent advances in whole genome expression analyses, namely RNA expression profiles produced by next-generation sequencing technology (RNA-Seq) allow comprehensive interrogation of transcribed sequences within cells or tissues, using very small amounts of total RNA. Advantages of this approach include an improved sensitivity to detect transcripts with low copy number, a significantly broader dynamic range resulting in enhanced sensitivity for detection of differential expression, detection of non-coding RNA's and isoform specific expression and identification of novel transcripts (Hertzano and Elkon, 2012; Scheffer et al., 2015). RNA expression profiles of tissues that are relevant to a particular trait are recognized as an important aspect of systematic genetic analysis of these traits. In addition, transcriptional data may be used in genetic mapping of functional genetic variants where large intervals that include multiple genes are identified, and often are difficult to narrow down to a specific gene of interest. Expression profiles of the specialized tissues were shown to be instrumental in narrowing the researchers' focus on genes that are functionally relevant to the trait (Vahedi et al., 2010). Last, regulatory non-coding RNAs display tissue-specific expression profiles and contribute to tissue patterning and to the control of different cellular programs, such as cell proliferation, differentiation, migration, or apoptosis. They have recently been implicated in the development of sensory organs and disorders (Conte et al., 2013). A recent paper focused on next generation sequencing of miRNAs in the mouse inner ear (Rudnicki et al., 2014), but a transcriptional inventory of long non-coding RNAs such as long intervening non-coding (lincRNAs) of the inner ear still remains incomplete. Thus, the lack of a well-characterized and in-depth analysis of the transcriptome of the human inner ear remains an impediment to progress in the field of hearing loss.

In this manuscript we describe, for the first time, RNA-Sequencing of the cochlea, ampulla, saccule and utricle of the adult human inner ear derived from fresh surgically resected tissue from four adult donor patients with normal hearing. We compare it to the transcriptome of 32 other human tissues, and focus on classes of RNAs potentially relevant to human hearing, such as specific mRNA isoforms and long non-coding RNAs. These data provide a rich, freely-accessible resource for understanding the inner ear and may prove useful as a resource for prioritization of candidate genes for the study of hearing loss.

2. Material and methods

2.1. Sample collection

Specimens were collected by trans-labyrinthine and trans-cochlear approaches to tumors of the skull base of three donor patients, where hearing preservation was not an option. The six specimens are as following: Individual 1: Ampulla, Utricle and Cochlea_3; Individual 2: Saccule and Cochlea_1; Individual 3: Cochlea_2. The trans-labyrinthine approach provides access to the structures of the vestibular labyrinth including the ampulla of the semicircular canals and the utricle and saccule. Extension of the inferior dissection of the internal auditory canal provides direct access to the cochlear lumen and easy removal of the cochlear duct. Tissues were removed under microscopic vision and immediately placed in Trizol (TRIzol (Life Technologies, Carlsbad, CA)) and RNA was extracted following the manufacturer's instruction. Samples were collected from three patients between the ages of 45 and 60 years old after obtaining informed consent. The experimental protocol and informed consent were approved by the institutional review board of St. Vincent's Medical Center, Los Angeles CA.

2.2. RNA-preparation and RNA-Seq

10–50 ng input of total RNA extracted from inner ear tissues was used for Ovation Total RNA (NuGEN, San Carlos, CA), followed by sample preparation (NEBNext, New England biolabs, Ipswish, MA). For the 32 other human tissues, total RNA was purchased from Asterand (Detroit, MI). RNA-Seq libraries were prepared for sequencing using Illumina's TruSeq mRNA Sample Preparation Kit (v2) using 0.1–1 ug total RNA input, following the manufacturer's protocol (Illumina Inc, San Diego, USA). All libraries were unstranded and rRNA depletion was not done. Sequencing was done by 104 bp paired-end sequencing on a HiSeq 2000 instrument (Illumina Inc, San Diego, USA), or 50 bp paired-end sequencing for the non-inner ear tissues. Inner ear samples were not barcoded and each ran on a single lane (18 pM). Other tissue samples were indexed and 4 samples were pooled on a flow cell, 20–22 pM. PhiX was spiked in on each lane (at the same pM).

2.3. RNA-Seq analysis and reference data

Analysis was performed in parallel by members of two groups (I.S. and Y.H.). Quality control (QC) of the unaligned data were done using FastQC (Andrews, 2010), and based on mean quality by cycle and the base frequency report (to detect skewing), data were trimmed.

Inner ear samples were aligned with RNAstar v2.3.0.1 after trimming to 75 bp based on QC (Dobin et al., 2013). A STAR reference was built using human genome annotation from ENCODE (Bernstein et al., 2012) with −sjdbGTFfile option, and setting −sjdbOverhang to 74 bp (as recommended for 75 bp reads). STAR alignment was performed setting the maximum number of mismatches to 5, with −outSAMstrandField intronMotif and

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