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Utilizing Single Cell RNA-Sequencing to Implicate Cell Types and Therapeutic Targets for SSNHL in the Adult Cochlea

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Abstract

OBJECTIVE: To identify genes implicated in sudden sensorineural hearing loss (SSNHL) and localize their expression in the cochlea to further explore potential pathogenic mechanisms and therapeutic targets.

STUDY DESIGN: Systematic literature review and bioinformatics analysis

DATA SOURCES: The following sources were searched from inception through July 2, 2020: PubMed-NCBI, MEDLINE, Embase, CINAHL, Cochrane Library, ClinicalTrials.gov, OpenGrey, GreyNet, GreyLiterature Report, and European Union Clinical Trials Registry. PubMed-NCBI and MEDLINE were additionally searched for human temporal bone histopathologic studies related to SSNHL.

METHODS: Literature review of candidate SSNHL genes was conducted according to PRISMA guidelines. Existing temporal bone studies from SSNHL patients were analyzed to identify the most commonly affected inner ear structures. Previously published single-cell and single-nucleus RNA-Seq datasets of the adult mouse stria vascularis, as well as postnatal day 7 and 15 mouse cochlear hair cells and supporting cells, were utilized for localization of the SSNHL-related genes curated through literature review.

CONCLUSIONS: We report 92 unique single nucleotide polymorphisms (SNPs) in 76 different genes that have been investigated in relation to SSNHL in the literature. We demonstrate that a subset of these genes are expressed by cell types in the adult mouse stria vascularis and organ of Corti, consistent with findings from temporal bone studies in human subjects with SSNHL.

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We highlight several potential genetic targets relevant to current and possible future SSNHL treatments.

Keywords

sudden sensorineural hearing loss; single nucleotide polymorphism; RNA-Seq; cochlea; temporal bone

INTRODUCTION

Sudden sensorineural hearing loss (SSNHL) is most commonly defined as a hearing loss of at least 30 decibels that spans a minimum of three contiguous audiometric frequencies and rapidly develops within a 72-hour period.[1] The estimated incidence of SSNHL in the U.S. has been reported to be between 5 to 27 per 100,000 people per year [2,3] with an average of over 66,000 new cases per year.[3] While some SSNHL cases can be attributed to an identifiable etiology, as high as 71% of cases remain idiopathic.[4] Multiple studies have analyzed structures of the inner ear in patients with SSNHL, and have reported abnormalities in various cell types.[5–11] However, the exact pathogenic mechanisms underlying SSNHL are not yet well characterized.

A growing body of research has focused on identifying factors implicated in the disease process, including genetic susceptibility. Over 65 potential genetic targets have been investigated in relationship to SSNHL to date[12], however, the expression of these candidate genes in cochlear cell types remains largely undefined. This study aimed to synthesize the available literature on genes and single nucleotide polymorphisms (SNPs) implicated in SSNHL. We reviewed the literature on human temporal bone histopathology studies to identify specific inner ear structures that have been most implicated in SSNHL to guide our gene localization. We then aimed to localize expression of the potential candidate SSNHL genes utilizing recently published single-cell and single-nucleus RNA-sequencing datasets from the adult mouse stria vascularis, as well as other published single-cell datasets from the cochlea, in order to provide greater insight into the mechanisms and potential anatomic locations of SSNHL pathogenesis.

METHODS

Literature Review

Systematic Review of SSNHL-Implicated Genes—A systematic review of genes implicated in SSNHL was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) reporting guidelines.[13] The following databases and grey literature sources were searched from inception through July 2, 2020: PubMed-NCBI, MEDLINE, Embase, CINAHL, Cochrane Library, ClinicalTrials.gov, OpenGrey, GreyNet, GreyLiterature Report, and European Union Clinical Trials Registry. No language restriction was employed. The following search terms were utilized: *sudden hearing loss* AND *polymorphism* (text words), *sudden hearing loss* AND *genes* (text words), *sudden deafness* (text words), and *hearing loss, sudden/ge [Genetics]* (MeSH term). Article titles and abstracts were screened for eligibility before full-text articles were obtained and

assessed for inclusion. Additional relevant articles were identified through reference lists of included studies or through manual searches, and were used to contextualize our findings. Data from included studies was extracted and compiled in a standardized electronic data collection sheet. The primary outcome of interest was the gene(s) or polymorphism(s) investigated in relation to SSNHL.

The strength of clinical data was graded independently by two authors according to the following rating scheme for individual studies, modified from the Oxford Centre for Evidence-Based Medicine levels of evidence: level 1, randomized clinical trials or systematic reviews with meta-analysis; level 2, controlled trials without randomization or prospective comparative cohort trials; level 3, case-control studies or retrospective cohort studies; level 4, case series with or without intervention or cross-sectional studies; level 5, opinion of respected authorities or case reports. The Newcastle Ottawa Scale was used to assess risk of bias for case-control studies, where studies receiving a score of greater than equal or to 7 were considered high quality and a low risk of bias.[14] The Joanna Briggs Institute Critical Appraisal Tools for analytical cross-sectional studies and case reports were utilized to assess risk of bias for each respective study type.[15,16]

Curation of SSNHL Gene List—The results of our literature search were compiled to create a list of SSNHL-implicated genes. We detailed which genes were supported by the literature to have a significant association with SSNHL versus those that were not found to have a significant association (Supplemental Digital Content, Tables 1–2). Genes were deemed to have a "significant association" if studies reported the gene to have statistically significant differential expression in SSNHL patients compared to controls. Genes deemed to have "no significant association" were reported in studies but were not found to have statistically significant differential expression between SSNHL patients and controls. The final curated list of SSNHL-implicated genes included all genes regardless of association and was utilized for cross-referencing with RNA-sequencing datasets as further described below.

Temporal Bone Histopathology Literature Review and Analysis—A supplemental literature review of temporal bone histopathology studies relating to SSNHL cases was also performed through a similar search method as described above. The MEDLINE and Pubmed-NCBI databases were searched from inception through January 2, 2021. The following search terms were utilized: *sudden hearing loss* AND *histopathology* (text words), *sudden hearing loss* AND *temporal bone* (text words). The processes for identifying eligible studies and data collection were conducted as described above.

Temporal bone histopathology analyses were performed by combining results from published studies evaluating temporal bone samples of individuals with SSNHL.[5–10] The data consisted of 71 ears (68 patients) with SSNHL and were standardized into a table by coding either "1" or "0" for the presence of abnormality (1=abnormal) in the following inner ear structures: hair cells, supporting cells, tectorial membrane, stria vascularis, spiral ganglion cells, and spiral limbus (Supplemental Digital Content, Table 3). Organ of Corti abnormalities were defined as loss of hair cells and/or supporting cells compared to control subjects. Tectorial membrane and stria vascularis abnormalities were defined as the presence

of degeneration or atrophy of these structures compared to controls. Spiral ganglion cells were analyzed by the method described by Otte et al.[17] The raw spiral ganglion cell counts from the Khetarpal et al.[8] data were determined to be abnormal if the measured number of spiral ganglion cells was less than the mean minus one standard deviation (n=9; mean = 23,815 cells; standard deviation = 3797) of measured spiral ganglion cells in healthy, control ears from Ungar et al.[11] The finalized data was compiled into a single spreadsheet and the number of abnormal cell type findings was divided by the total number of analyzed subjects for each ear structure to give a percentage of subjects with abnormal findings. Subjects from studies that did not report findings for a particular cell type were excluded from the final counts and analysis for that cell type.

Bioinformatics

Data and Software Availability—Our curated list of SSNHL-implicated genes was cross-referenced against the following previously published datasets. Single cell and single nucleus RNA-Seq datasets of postnatal day 30 (P30) mouse stria vascularis[18] were utilized (GEO Accession ID: GSE136196) (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE136196) and are available through the gene Expression Analysis Resource (gEAR) Portal, a website for visualization and comparative analysis of multi-omic data, with an emphasis on hearing research (https://umgear.org//index.html?layout_id=b50cae7a). Single cell RNA-Seq dataset from the P7 developing mouse cochlea including IHCs, OHCs, and supporting cells including both inner phalangeal, pillar, and Deiters cells [19] was utilized (GEO Accession ID: GSE137299) and are available through gEAR (https://umgear.org//index.html?layout_id=f7baf4ea). Finally, previously published single cell RNA-Seq datasets of postnatal day 15 (P15) inner hair cells (IHCs), outer hair cells (OHCs), and Deiters cells[20] were utilized (GEO Accession ID: GSE114157) (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE114157) and are available through the Molecular Otolaryngology and Renal Research Laboratories (MORL) (https://morlscrnaseq.org/). Previously published datasets from the P25–27 adult mouse spiral ganglion neurons[21] and from the adult human cochlea[22] were also utilized for additional gene localization analysis, included in the supplemental materials (Supplemental Digital Content, Figures 2–3).

Data Visualization

P30 SV scRNA-Seq & snRNA-Seq.: Previously published P30 SV scRNA-Seq and snRNA-Seq data were preprocessed by Scanpy (v1.5.1) with criteria as previously described.[18,23]

P7 IHCs, OHCs, and supporting cells.: Previously published normalized dataset from Kolla and colleagues [19] was processed by Scanpy (v1.5.1). “Rik” and “Gm-” genes were filtered in all downstream analysis. Cell clustering and annotation was performed using modularity-based clustering with Leiden algorithm (resolution=0.8) implemented in Scanpy. Heatmaps were plotted by Seaborn (v0.10.1).

P15 IHCs, OHCs, and Deiters cells.: Data expression matrix from previously published P15 IHC, OHC, and Deiters cell single cell RNA-Seq dataset was normalized by Scanpy (v1.5.1) using the function pp.normalize_total with parameter “exclude_highly_expressed”

set as True. Normalized dataset was then logarithmized by the function pp.log1p. Heatmaps were plotted by Seaborn (v0.10.1).

Information on the data visualization from the P25–27 spiral ganglion neurons and the human cochlea datasets are included as supplements (Supplemental Digital Content, Figures 2–3).

RESULTS

Systematic Literature Review of SSNHL-Implicated Genes

A PRISMA-based systematic literature review was conducted to curate a list of SSNHL-implicated genes (see Fig. 1 for complete search strategy). Database searching initially identified 254 articles of potential relevance. After removal of duplicate texts, 161 articles remained and were screened for eligibility. Exclusion criteria removed studies that did not relate to SSNHL or did not include genetic analysis (n=7), full-text articles in a foreign language (n=5), and other systematic reviews (n=7). In total, 63 studies fully satisfied the inclusion criteria and were included in the final review. The majority of studies (87%) were rated to be of clinical strength level 3, with the remaining few rated to be level 2 or level 4, in accordance with the modified Oxford Centre for Evidence-Based Medicine grading scale described above. When assessing risk of bias, all included case-control studies received a score of 7 or greater on the Newcastle Ottawa Scale, indicating high quality and low risk of bias (Supplemental Digital Content, Table 4A). The single analytical cross-sectional study and the four case reports included in the review were evaluated according to the Joanna Briggs Institute Critical Appraisal methodology and met all criteria within each assessment tool. (Supplemental Digital Content, Table 4B).

The 63 included studies involved 6,165 subjects with SSNHL (52.1% male, 47.9% female, mean age of 50.1f ± 12.16). Additional demographics and characteristics of included subjects are detailed in Table 1. Not all studies consistently reported on features related to past medical history and associated symptoms of SSNHL; the table values only reflect data from studies that did report on those measures. Of the 63 studies, 25.4% recorded a history of hypertension and diabetes mellitus, 20.6% reported on dyslipidemia, and 15.9% reported on smoking status. With regard to SSNHL-associated symptoms, tinnitus, vertigo, and hearing recovery were tracked in 31.7%, 36.5%, and 50.8% of studies, respectively. Many studies employed varied methodologies to categorize levels of hearing recovery. The full list of included studies along with study design, population, subject number, and investigated genes is detailed in Supplemental Digital Content, Table 5.

Literature Review of Temporal Bone Studies in SSNHL

Six temporal bone studies were identified for inclusion that reported histopathological findings in patients with SSNHL. These studies report 71 ears (68 subjects) identified, with 38% female (n = 26), average age of onset 48.3 years (standard deviation = 22.1 years), and average age of death 60.1 years (n = 56 subjects, standard deviation = 17.3 years) (Table 1). The most common structural abnormalities were seen in the supporting cells (87%), hair

cells (86%), and stria vascularis (75%), followed by spiral ganglion cells (56%), tectorial membrane (51%), and spiral limbus (49%) (Fig. 2).

Candidate Genes Implicated in SSNHL

We present a list of 92 unique SNPs in 76 different genes that have been investigated in relation to SSNHL. 47 genes were found by at least one study to have a significant association with SSNHL, while the remaining 29 were never found to have a significant association. For 9 of the 47 genes of significance, there was conflicting evidence in the literature where at least one additional study did not find a significant relation to SSNHL for the same gene. Notably, 15 of the 76 total genes had multiple SNPs investigated across different studies, and the relationship to SSNHL was found to differ between some of the distinct SNPs. We provide documentation of each investigated gene and SNP along with their respective supporting and non-supporting studies (Supplemental Digital Content, Tables 1–2).

Gene Target Association with SSNHL Susceptibility

The majority of genetic targets found to have a significant association with SSNHL were suggested to play a role in disease susceptibility. Specifically, 23 genes had at least one SNP that was correlated with an increased risk of SSNHL. Increased risk was determined by a significantly higher SNP frequency observed in affected SSNHL subjects compared to controls. In contrast, the following seven genes had at least one SNP correlated with a decreased risk: *APOE*, *DNMT1*, *FCRL3*, *GRHL2*, *GPX3, HSPA1A*, *SERPINE1*[24–30] (Supplemental Digital Content, Table 1). Decreased risk was inferred by the observation of a significantly lower SNP frequency in SSNHL subjects compared to controls, or by a significantly higher SNP frequency in controls compared to SSNHL subjects. Interestingly, there were two genes, *DNMT1* and *FCRL3*, for which specific genotypes in different SNPs of each gene were found to have opposite associations with SSNHL risk.[24,26]

Localization of Candidate SSNHL Genes to Regions and Cell Types in the Cochlea with Single Cell Resolution.

The cochlea is composed of a diverse collection of intricately arranged cell types that function together to enable hearing. The advent of publicly available single cell RNA-sequencing datasets from the mammalian cochlea provides the opportunity to characterize candidate gene expression in silico at the single cell level. Our list of SSNHL-implicated genes was cross-referenced against previously published single cell and single nucleus RNA-seq datasets from the postnatal day 30 (P30) mouse stria vascularis (SV).[18] Heatmaps demonstrating SSNHL-implicated gene expression amongst SV cell types are shown in both the single cell (Fig. 3A) and single nucleus (Fig. 3B). For each heatmap, gene expression is displayed along the vertical axis while SV cell types are displayed along the horizontal axis. Expression is in normalized transcript counts with an increasingly dark purple color indicating a higher number of transcripts in a given cell. Genes that demonstrate enriched expression in the SV include *Sod1, Gpx3,Mif, Gjb2, Nr3c1, Clock, Gpx1, Sod2*, and *Nr3c2*.

By cross-referencing SSNHL-implicated genes against a recently published P7 mouse organ of Corti scRNA-Seq dataset,[19] heatmaps depicting inner and outer hair cells, pillar cells,

and Deiters cells demonstrate enrichment for *Sod1, Hspa1a, Gpx1*, and *Mif*. Transcripts for *Apoe*, *Gjb2*, and *Pon2* are enriched in pillar and Deiters cells (Fig. 4). Finally, in examining a previously published P15 scRNA-Seq dataset of inner and outer hair cells as well as Deiter cells,[20] heatmaps demonstrate expression of *Sod1, Gpx1,Mif, Gjb2*, *Nr3c1, Nr3c2,* and *Gpx3* transcripts (Fig. 5).

Violin plots further demonstrate the differential expression of glutathione peroxidase isoforms amongst cochlear cell types (Supplemental Digital Content, Fig. 1). For each violin plot, cell types within a tissue (organ of Corti or SV) are displayed along the horizontal axis and expression level in normalized counts is displayed along the vertical axis. The wider the violin, the more cells at a given level of expression. Of note, in the SV datasets, *Gpx3* appears to be preferentially expressed amongst SV marginal, intermediate, and basal cells over *Gpx1* (Supplemental Digital Content, Fig. 1A–B) compared to the relative enrichment of *Gpx1* transcripts over *Gpx3* transcripts amongst cells in the Organ of Corti (Supplemental Digital Content, Fig. 1C–D).

Gene localization results utilizing the P25–27 spiral ganglion neurons and human cochlea datasets are available in the supplemental materials (Supplemental Digital Content, Figures 2–3).

DISCUSSION

This study provides a novel, comprehensive report of 92 SNPs in 76 distinct genes that have been investigated as candidate SSNHL genetic targets. Our review of temporal bone histopathology studies in SSNHL cases demonstrates that the organ of Corti (supporting cells and hair cells) and the stria vascularis (SV) are the most commonly affected structures. Lastly, our analysis localizes expression of SSNHL-implicated genes to the SV and organ of Corti based upon single-cell and single-nucleus RNA sequencing datasets. This data demonstrates that genes implicated in SSNHL, some of which were previously identified through peripheral blood assays, are expressed by relevant cell types and tissues in the cochlea. This localization establishes a rationale for examining the role of these genes in mediating SSNHL pathogenesis.

Human Temporal Bone Studies Identify Inner Ear Structures Implicated in SSNHL Pathogenesis

Our review of published human temporal bone studies summarizes available data regarding the location of cochlear pathology in SSNHL. These studies identify multiple abnormalities in inner ear structures including the supporting cells, hair cells, stria vascularis (SV), spiral ganglion cells, tectorial membrane, and spiral limbus. Importantly, in addition to hair cells and supporting cells, these studies reveal that the SV is the next most commonly involved structure after the organ of Corti. While much attention has been focused on hair cell loss, these analyses suggest additional attention to the role of the SV in SSNHL may be warranted. With this in mind, leveraging knowledge from published single cell RNA-sequencing datasets offers the opportunity to begin to understand the role that the SV may play in SSNHL by identifying involved cell types and possibly by identifying critical regionally specific cell signaling pathways.

Localization of SSNHL-Implicated Genes to Inner Ear Cell Types

Cross-referencing of SSNHL-implicated genes against single cell RNA-sequencing datasets suggests that many genes related to hearing loss susceptibility are expressed by critical cell types in the inner ear. Several genes that demonstrate prominent expression in the cochlea include superoxide dismutase 1 (*Sod1)* and 2 (*Sod2)*, as well as macrophage migration inhibitory factor (*Mif)* (Fig. 3–6). Superoxide dismutase 1 (*Sod1*) and 2 (*Sod2*) encode a copper- and zinc-binding catalase and a manganese-binding catalase, respectively, that are responsible for the destruction of free superoxide radicals and have been implicated in SSNHL.[31,32] *Sod1* has previously been shown to be expressed in the SV and to a lesser extent the organ of Corti in mice and rats, with evidence of increased expression in the setting of oxidative stress.[33,34] *Sod1* knockout mice have been shown to be more susceptible to noise-induced permanent threshold shifts compared to control wildtype mice.[35] Overexpression of both *Sod1*and *Sod2* in mouse models has been implicated in mitigation of aminoglycoside ototoxicity.[36–38] Our finding that *Sod1* and *Sod2* are prominently expressed in the cochlea further complements the existing literature that implicates *Sod1* and *Sod2* in hearing loss pathogenesis.

Macrophage migration inhibitory factor (*Mif*) was another gene found to be prominently expressed in the cochlea — again consistent with previous studies that have indicated a role for *Mif* in hearing loss.[39] *Mif* has been shown to be expressed in the SV, spiral ligament, spiral limbus, organ of Corti, and Reissner's membrane,[40] and its expression appears to be increased in the setting of experimentally-induced inflammation with LPS treatment in mice.[41] *Mif* knockout mice appear to be more susceptible to age-related hearing loss than wildtype mice.[40] Some evidence furthermore suggests that SSNHL patients demonstrate higher levels of circulating MIF when compared to normal-hearing patients, and that higher circulating MIF levels and possibly *MIF*-related SNPs may impact steroid-responsiveness of SSNHL.[39,42] Together, these observations suggest that genes implicated in hearing los susceptibility are involved at the tissue and cellular level in the cochlea in SSNHL.

Implication of Gene Expression Localization for Understanding SSNHL Therapeutic Targets

Our analysis localizes several genes relevant to SSNHL treatment — such as *Gpx1*, *Gpx3*, *Nr3c1,* and *Nr3c2* — to cochlear cell types, contributing to our understanding of both current and potential future therapeutic targets. Glutathione peroxidases, *Gpx1* and *Gpx3*, have been shown to play a role in otoprotection from oxidative damage with loss of glutathione peroxidases (GPx) associated with increased hearing loss susceptibility due to noise, aminoglycosides, and cisplatin [43–45] as well as SSNHL.[28,32] Furthermore, increased expression of GPx has been associated with protection of hair cells in the presence of cisplatin,[37,46] and administration of a GPx mimetic, ebselen, appears to result in some degree of otoprotection after both cisplatin- and noise-induced hearing loss.[45,47–49] Interestingly, ebselen (SPI-1005) is currently being investigated as a treatment for Meniere's disease (https://www.clinicaltrials.gov/ct2/show/NCT03325790), a disease that can often present with sudden changes in hearing. Our analysis suggests that glutathione peroxidase isoforms (*Gpx1* and *Gpx3*) are differentially expressed amongst cochlear cell types with preferential expression of *Gpx3* in the SV, with notably prominent expression in SV

marginal cells (Supplemental Digital Content, Fig. 1). Whether drugs targeting GPx and potentially its different isoforms in the cochlea might be effective in the treatment of SSNHL remains to be evaluated, and presents an avenue for future research.

*Nr3c1*and *Nr3c2* encode glucocorticoid and mineralocorticoid receptors, respectively. Previous studies have localized glucocorticoid receptors to inner ear cell types in both mice and humans.[50,51] Consistent with these observations, we demonstrate that *Nr3c1* and *Nr3c2* expression is localized to both the organ of Corti and the SV. Since steroids are largely the only approved pharmacologic treatment option for patients with SSNHL[1], understanding the localization of these receptors to cochlear cell types is relevant to understanding the effect of steroids in the inner ear. Steroids are thought to interact with both glucocorticoid and mineralocorticoid receptors.[52–57] Trune and colleagues have suggested that, in the setting of autoimmune inner ear disease, the effects of steroids mediated by mineralocorticoid action may partially account for the steroid-responsiveness of hearing loss in these patients.[52,58] With this assertion in mind, it is interesting that certain SNPs of *NR3C1*, which may alter glucocorticoid receptor expression, have been associated with poor glucocorticoid response in SSNHL.[59] Given these observations, it is critical to further understand cell types and tissues that appear to be affected in humans with SSNHL in order to better appreciate the mechanisms underlying clinical responses to therapeutics.

Limitations

There are a few limitations that are important to acknowledge when discussing our findings. First, associations between SSNHL and many of the candidate genes were based only on differential gene expression or detection of gene-related SNPs in peripheral blood samples rather than local inner ear tissue samples. Furthermore, many of the candidate genes were selected for investigation based upon proposed pathways involved in SSNHL pathogenesis such as inflammation, oxidative stress, and thrombosis, regardless of existing supporting evidence. All of the studies included in the systematic literature review were observational, and thus a causal relationship between the identified candidate gene targets and SSNHL cannot be fully evaluated. We additionally recognize that differential gene expression via RNA-sequencing does not directly establish functional significance of the localized genes. Nonetheless, by synthesizing the available literature and localizing expression of the investigated genes to relevant regions and cell types in the mammalian cochlea, we make necessary initial steps towards characterizing the genetic and mechanistic underpinnings of SSNHL.

CONCLUSIONS

In summary, we present a novel report of the 96 potential genetic targets for SSNHL that have been investigated in the literature to date; 47 genes for which the association reached significance, and 23 genes of which were correlated with an increased risk of SSNHL. We localize these genes implicated in SSNHL to cochlear structures consistent with human temporal bone studies and to specific cochlear cell types, including those in the organ of Corti and in the SV. Importantly, we identify and localize gene expression relevant to current

treatments for SSNHL, contributing to our understanding of the fundamental role of these genes in the etiology of SSNHL and presenting avenues for further study.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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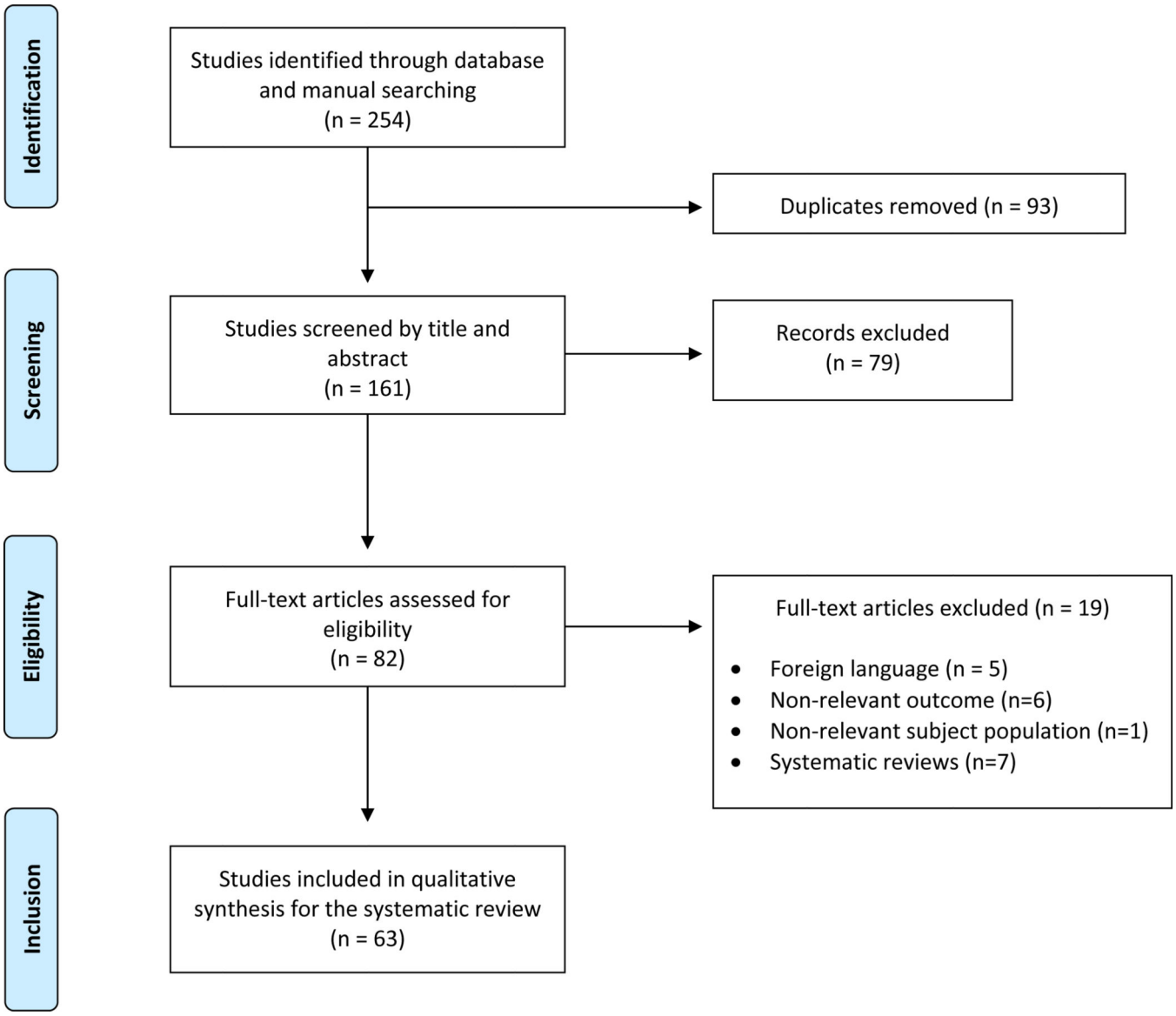

Figure 1. PRISMA Flow Diagram for Systematic Literature Review of SSNHL-Implicated Genes

Flow diagram illustrates the literature review and study selection process conducted according to PRISMA reporting guidelines for published studies investigating genes related to SSNHL. A total of 63 studies satisfied inclusion criteria and were included in the final review.

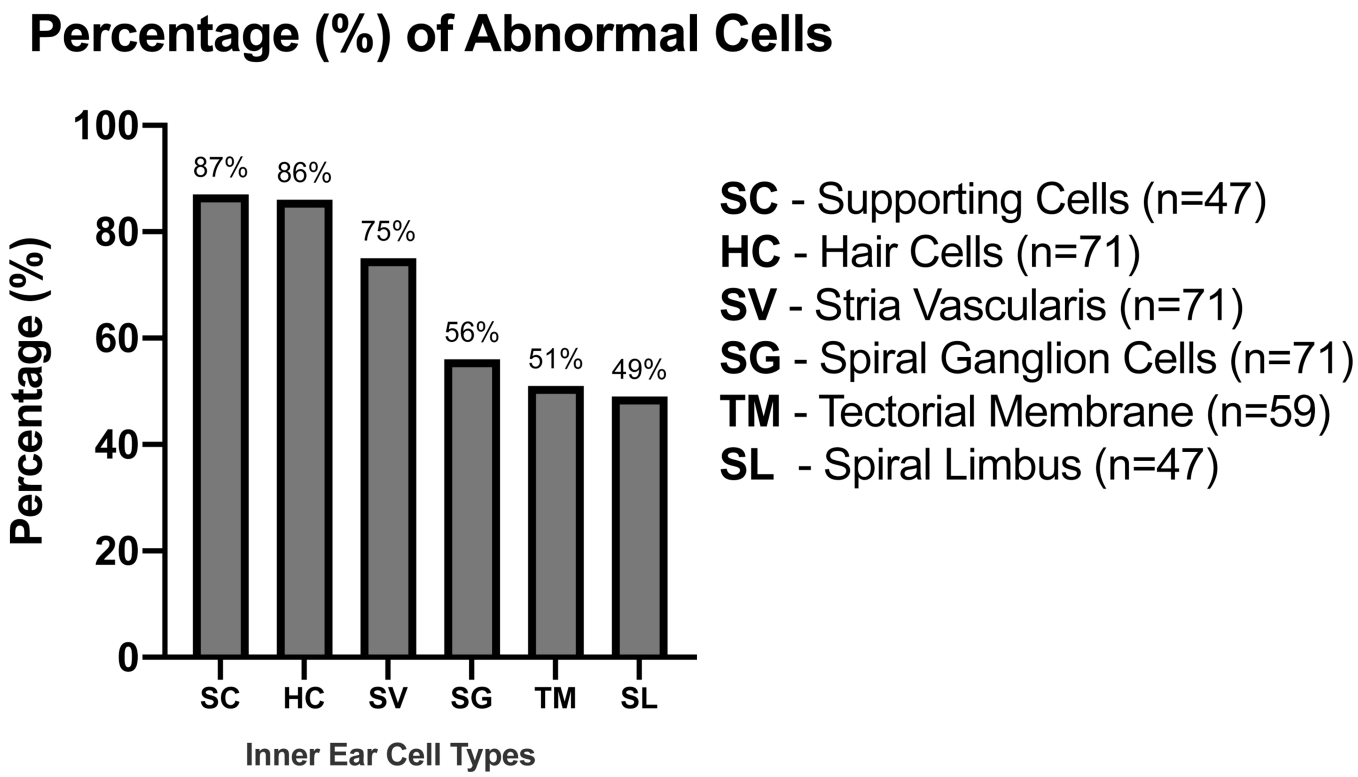

Figure 2. Distribution of Inner Ear Cell Type Abnormalities Identified in Human Temporal Bone Studies

Published temporal bone studies reporting histopathological findings from patients with SSNHL were analyzed to determine the distribution of inner ear cell types found to have structural abnormalities. Graph displays inner ear cell types along the horizontal axis and percentage of abnormal cells on the vertical axis. Cell types include supporting cells (SC), hair cells (HC), stria vascularis (SV), spiral ganglion (SG), tectorial membrane (TM), and spiral limbus (S). Percentage of abnormal cells was calculated by dividing the number of abnormal samples by the total number of analyzed subjects for each cell type. Subjects from studies that did not report findings for particular cell types were excluded from the analysis. Cell type abnormality was defined by criteria that varied by study and included metrics such as significantly decreased cell count and/or presence of structural degeneration or atrophy in SSNHL patient samples compared to controls. Cell type-specific definitions for abnormality are provided in the methods.

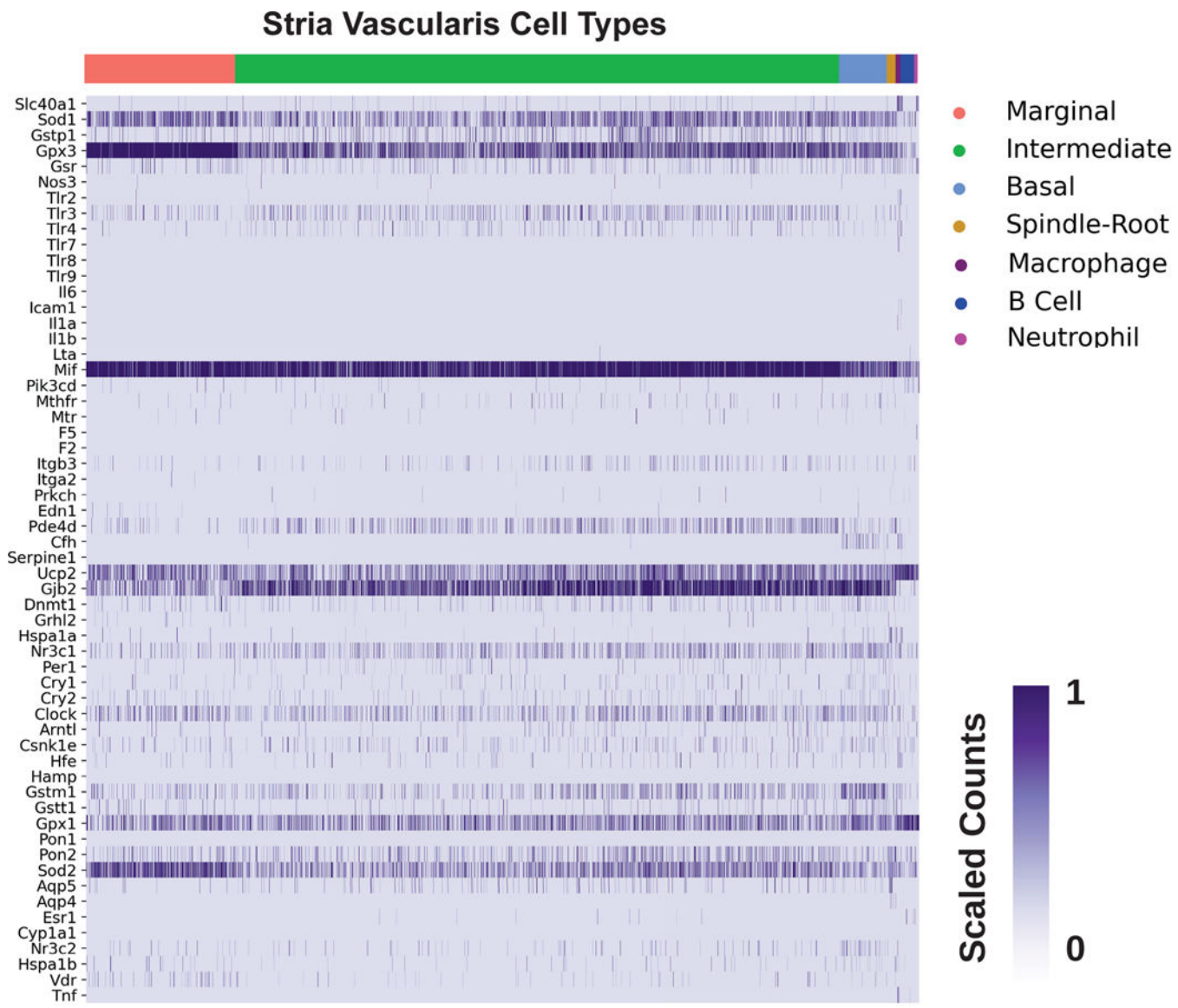

Figure 3A. Expression of SSNHL-investigated genes in the adult mouse stria vascularis utilizing a single cell RNA-Seq adult mouse stria vascularis dataset (Korrapati, Taukulis et al., 2019).

Heatmap displays cells along the horizontal axis with cell types grouped by color and SSNHL-investigated genes along the vertical axis. The darker the bar the more highly expressed the gene is in a given cell. Cell types include SV cell types (marginal cells in pink, intermediate cells in green, basal cells in light blue), cells at the boundary of the SV (spindle-root cells in gold, fibrocytes in light green), as well as immune cells (macrophages in purple, B cells in blue, and neutrophils in magenta). Spindle and root cell profiles were not distinguishable from each other in this dataset.

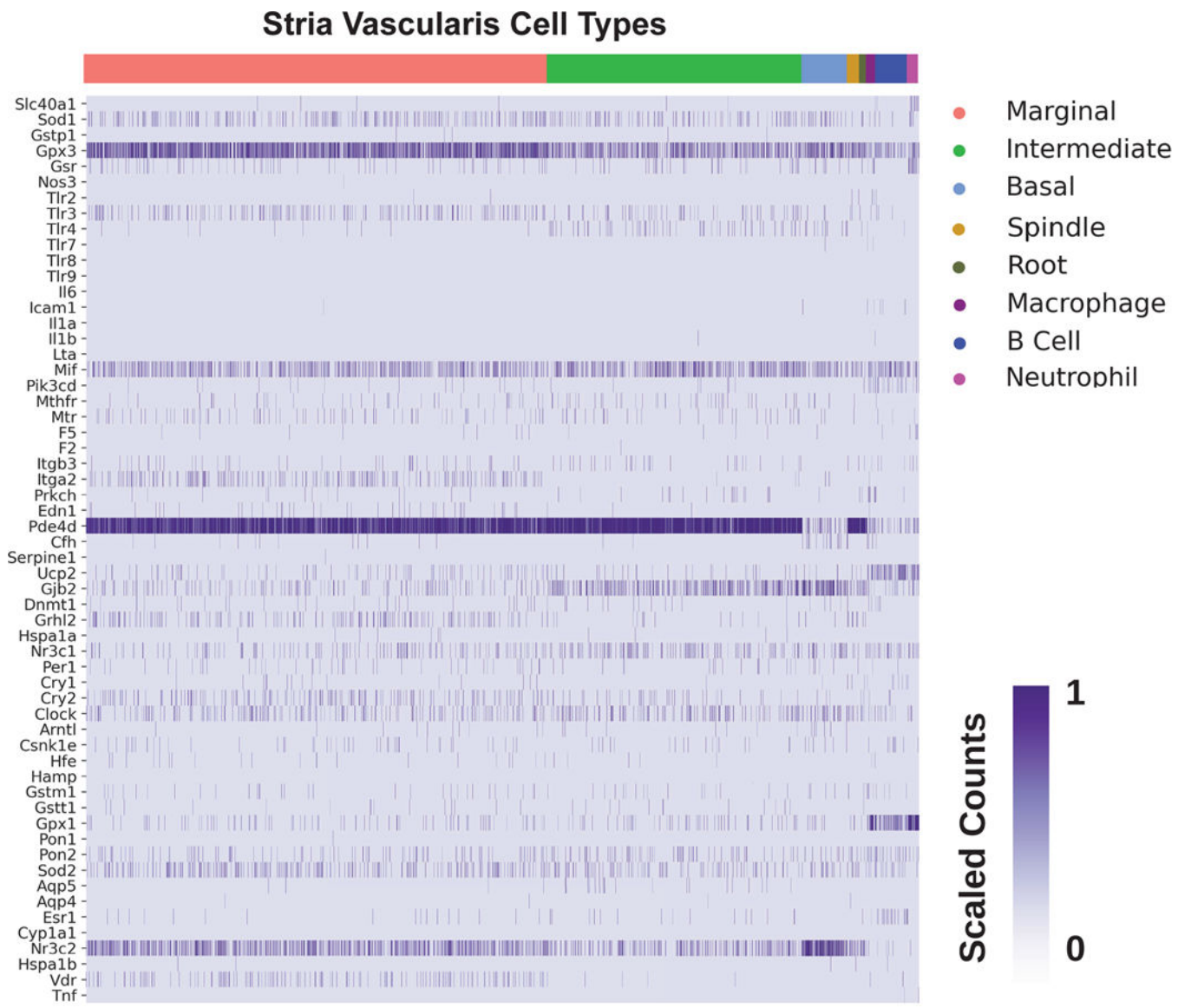

Figure 3B. Expression of SSNHL-investigated genes in the adult mouse stria vascularis utilizing a single nucleus RNA-Seq adult mouse stria vascularis dataset (Korrapati, Taukulis et al., 2019).

Heatmap displays cells along the horizontal axis with cell types grouped by color and SSNHL-investigated genes along the vertical axis. The darker the bar the more highly expressed the gene is in a given cell. Cell types include SV cell types (marginal cells in pink, intermediate cells in green, basal cells in light blue), cells at the boundary of the SV (spindle cells in gold, root cells in dark green, fibrocytes in light green), as well as immune cells (macrophages in purple, B cells in blue, and neutrophils in magenta).

Figure 4. Expression of SSNHL-investigated genes in P7 mouse organ of Corti utilizing a single cell RNA-Seq P7 mouse organ of Corti dataset (Kolla et al., 2020).

Heatmap displays cells along the horizontal axis with cell types grouped by color and SSNHL-investigated genes along the vertical axis. The darker the bar the more highly expressed the gene is in a given cell. Cell types in the organ of Corti analyzed for this heatmap include inner hair cells (IHC) in pastel green, outer hair cells (OHC) in gray, pillar cells in midnight blue, and Deiters cells in forest green.

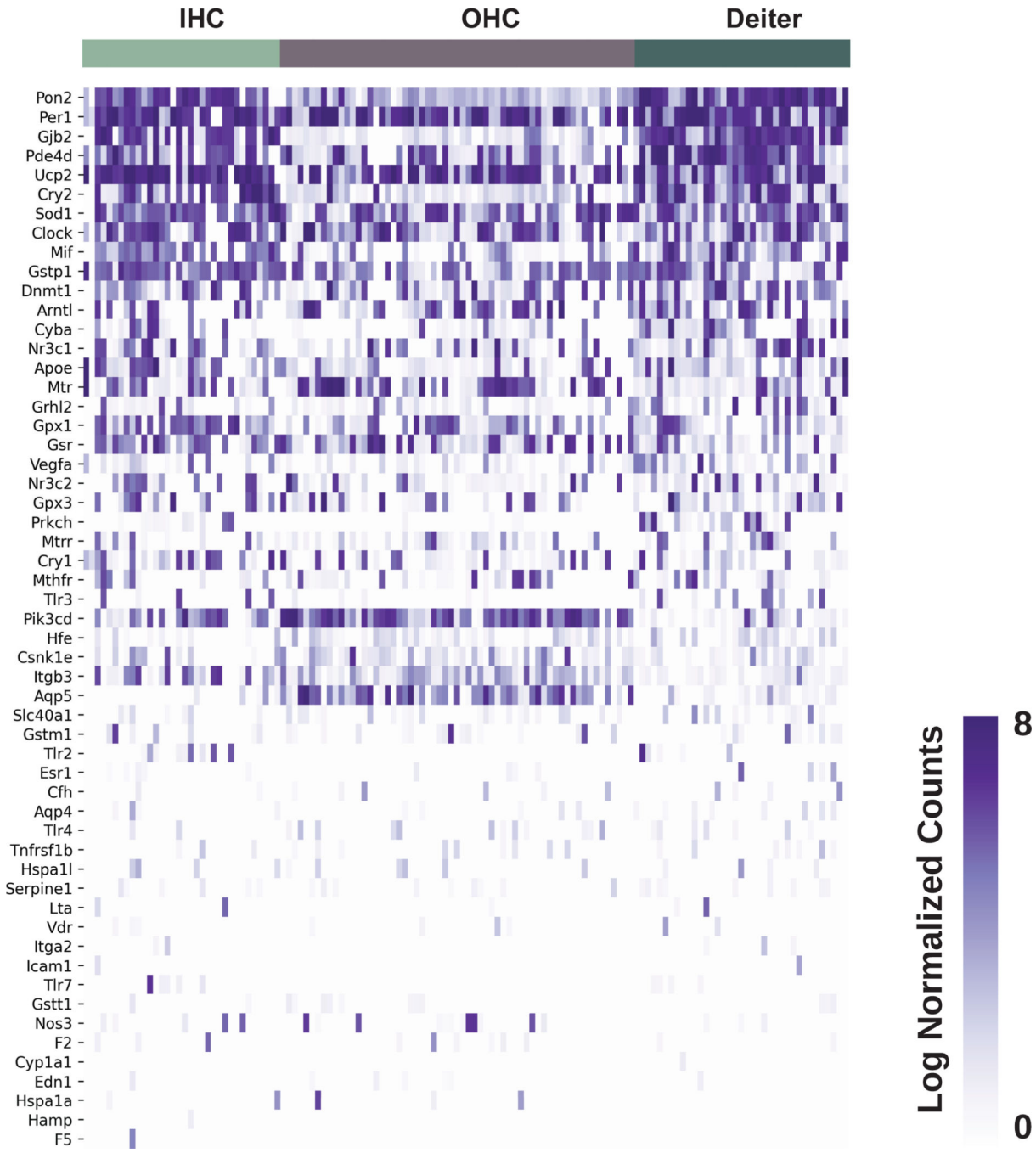

Figure 5. Expression of SSNHL-investigated genes in P15 mouse organ of Corti utilizing a single cell RNA-Seq P15 mouse organ of Corti dataset (Ranum et al., 2019).

Heatmap displays cells along the horizontal axis with cell types grouped by color and SSNHL-investigated genes along the vertical axis. The darker the bar the more highly expressed the gene is in a given cell. Cell types in the organ of Corti available for analysis include inner hair cells (IHC) in blue, outer hair cells (OHC) in gold, and Deiters cells in green.

Table 1.

Subject Demographics of Included SSNHL and Temporal Bone Studies

	N (Average %)	# Studies Reporting Characteristic
SSNHL Study Demographics		
Total # subjects	6,165	63
Male	3,213 (52.1%)	63
Female	2,952 (47.9%)	63
Mean Age (years)	50.1 ± 12.16	63
Unilateral SSNHL	6,145 (99.7%)	63
Bilateral SSNHL	20 (0.3%)	63
Tinnitus	1252 (69.1%)	20
Vertigo	572 (34.4%)	23
Hearing recovery	1254 (54.9%)	32
DM	236 (15.2%)	16
Hypertension	365 (22.9%)	16
Dyslipidemia	276 (22.3%)	13
Smoking	325 (28.5%)	10
Temporal Bone Study Demographics		
Total # subjects	68	6
Male	42 (61.8%)	6
Female	26 (38.2%)	6
Mean age of SSNHL onset (years)	48.3 ± 22.1	6
Mean age of death (years)	60.1 ± 17.3	6
SSNHL laterality, right ear	38 (55.9%)	6
SSNHL laterality, left ear	27 (39.7%)	6
SSNHL laterality, bilateral	3 (4.4%)	6