AGE-RELATED CELLULAR AND MOLECULAR CHANGES ASSOCIATED WITH VESTIBULAR SENSORY EPITHELIUM

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BACKGROUND

- Presbyastasis (ARVL) is the gradual loss of bilateral vestibular function accompanied by interruptions to visual and proprioceptive inputs leading to an increased risk of imbalance and is characterized by dizziness, vertigo, and falls which can be fatal.
- In adults older than 70 years, there is a 40% decrease in sensory hair cell (HC) density in the cristae of the canals, 24% in the saccule, and 21% in the utricle.
- In addition to HC loss, morphological and functional changes have also been demonstrated in the remaining HCs.
- The damaging impact of such age-related vestibular sensory decline manifests itself in an exponential increase in geriatric dizziness and a subsequent higher prevalence of injurious falls leading to Presbyastasis.
- While many studies have focused on age-related hearing loss and cochlear HC degeneration, age-related vestibular HC degeneration and their contribution to age-related vestibular disorders remain elusive.
- Hence, in the current study, we focused on investigating the molecular and cellular changes associated with vestibular HC and supporting cell (SC) degeneration during physiological aging.

SIGNIFICANCE OF PROBLEM

- According to the National Center for Health Statistics (NCHS), the prevalence of balance impairment in the United States is 75.3% in the elderly with a total annual medical expenditure of $48.1 billion.
- Moreover, NIDCD reports that age-related falls account for 50% of all accidental deaths and it is the 6th leading cause of death in the elderly.
- Currently, available treatment options include vestibular rehabilitation therapy which comprises physical therapy, lifestyle changes, and medications to alleviate symptoms such as dizziness, vertigo, and spatial disorientation. However, there are no targeted treatment strategies to delay or prevent the onset of Presbyastasis highlighting the importance of identifying novel therapeutic targets.
- Evidence shows that there is an increased age-related morphological and functional decline in the vestibular system, but the driving factors and underlying mechanisms involved at the molecular and cellular level that lead to age-related pathophysiological changes in the vestibule are unknown.
- Therefore, understanding how the onset of age-related vestibular dysfunction correlates with cellular and molecular degeneration of the vestibular sensory epithelium may provide new insights into mechanisms underlying vestibular aging and developing targeted treatment strategies.

HYPOTHESIS

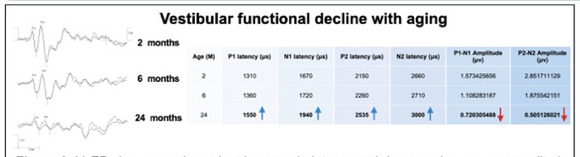
Age-related cellular and molecular changes lead to degeneration of the vestibular hair cells and supporting cells.

EXPERIMENTAL DESIGN AND METHODS

CBA/J mice aged between 2 and 24 months after birth were used for our experiments. We measured vestibular evoked potential (VsEP) at the system level to determine changes in vestibular function. At the cellular level, we examined changes in the morphology and ultrastructure using immunostaining (combined with confocal microscopy) and scanning electron microscopy. At the molecular level, we used single-cell RNA-sequencing (scRNA-seq) to examine changes in transcriptomes of HCs and SCs during aging. For scRNA-seq, vestibular sensory end organs of saccule, utricle, and crista were isolated. Each age group contains an equal number of males and females and a total of 100 mice were used to obtain 5 biological replicates for scRNA-seq. Droplet-based scRNA-seq was performed using the 10x Genomics chromium platform and raw data was processed by Cell Ranger to obtain count matrices. Downstream quality control, analysis, and visualization were performed in R using the Seurat package (4.3.0.1). mRNA expression will be validated by RNAscope.

RESULTS

Vestibular functional decline with aging



	Age (M)	P1 latency (μs)	N1 latency (μs)	P2 latency (μs)	N2 latency (μs)	P1-N1 Amplitude (μv)	P2-N2 Amplitude (μv)
2 months	2	1310	1670	2150	2660	1.573425656	2.851711129
6 months	6	1360	1720	2260	2710	1.108283187	1.875542151
24 months	24	1550 ↑	1940 ↑	2535 ↑	3000 ↑	0.720305488 ↓	0.505126021 ↓

Figure 1: VsEP shows age-dependent increase in latency and decrease in response amplitude indicating age-related vestibular functional decline in 2, 6-month and 24-month-old CBA/J mice aligning with previous findings.

Vestibular morphological decline with aging

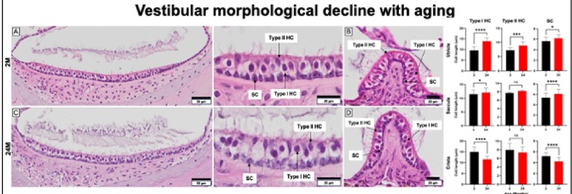


Figure 2: H&E staining analysis of utricle and saccule show age-related elongation of cell soma and conversely a reduction in cell soma in the crista. A shows the representative images and B shows the cell length quantifications.

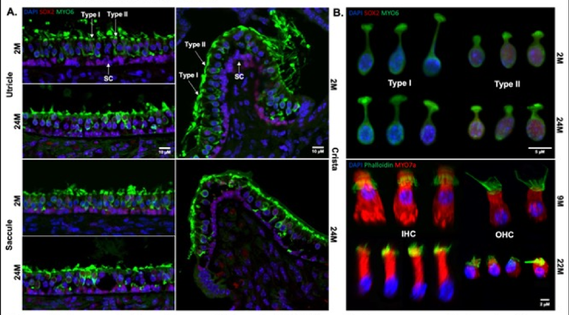


Figure 3: Immunostaining of young (2M) and old (24M) vestibular sensory epithelium depicts age-related alterations. A shows the representative images and B shows changes at the individual hair cell level.

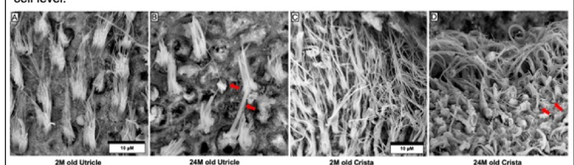


Figure 4: Scanning electron microscopy shows age-related loss of stereocilia hair bundles, bundle degeneration, and fusion in utricle and crista. Figures A and C depict young while B and D show old utricle and crista respectively.

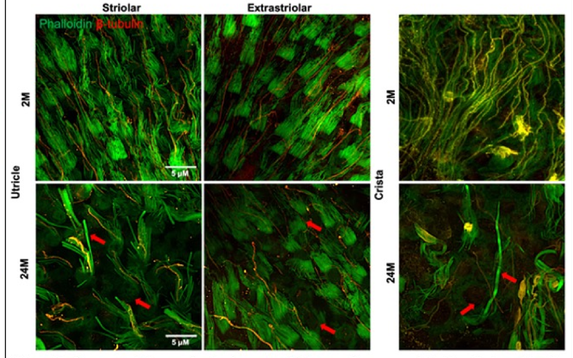


Figure 5: Super-resolution confocal microscopy further confirms age-related bundle loss, kinocilia degeneration, and bundle fusion in the utricle and crista. Aging crista and striolar regions of the utricle exhibit increased bundle loss, degeneration, and fusion in contrast to extrastriolar regions.

Transcriptomic changes associated with vestibular aging

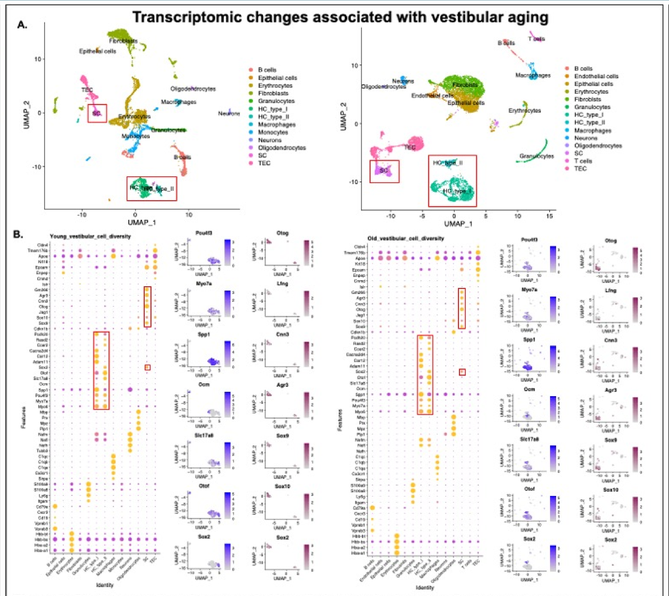


Figure 6: Cluster annotation of young and old vestibular samples based on the known marker gene expression analyzed by R. Hair cell and supporting cell clusters and marker genes are highlighted in red.

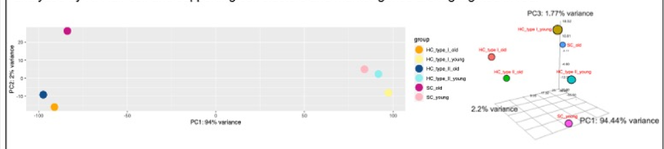


Figure 7: Principle component analysis of young and aged vestibular hair cells and supporting cells reveal a significant variance of 94% indicating how different the aged HC and SC are from the young HC and SC.

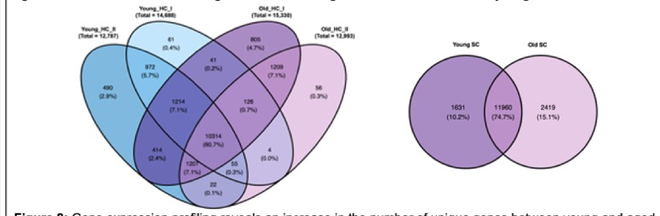


Figure 8: Gene expression profiling reveals an increase in the number of unique genes between young and aged type I hair cells and supporting cells and a decrease in the unique genes in type II hair cells respectively indicating age-related transcriptomic changes.

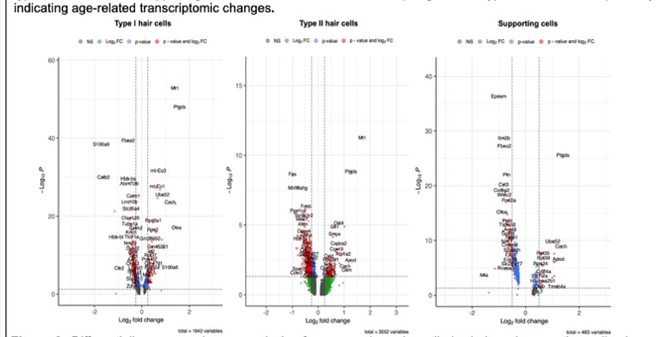


Figure 9: Differentially expressed gene analysis of young and aged vestibular hair and supporting cells shows alterations in the genes related to mechanotransduction, cell soma, cell adhesion, and various hallmark pathways of aging.

Transcriptomic changes associated with vestibular aging

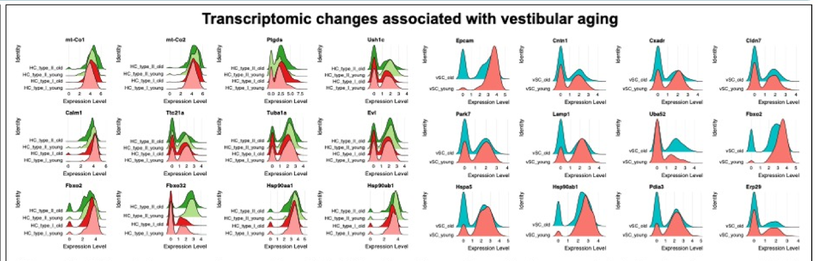


Figure 10: Differentially expressed genes of vestibular hair cells and supporting cells show age-related alterations in genes related to metabolism, mechanotransduction, cell adhesion, ubiquitination, autophagy, and proteostasis.

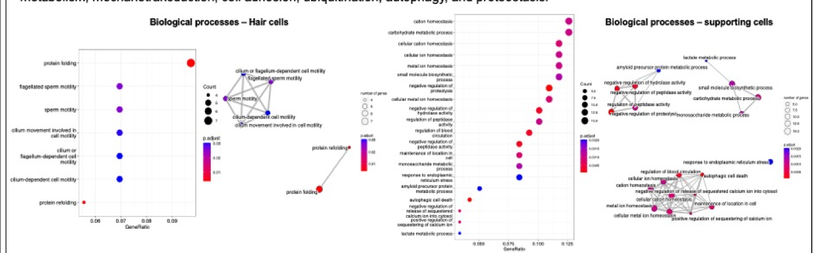


Figure 11: GO enrichment analysis further shows a significant downregulation in genes related to bundle motility, mechanotransduction, and proteostasis highlighting underlying mechanisms of hair cell aging. Aged supporting cells exhibit a notable decline in ion and fluid homeostasis for optimal hair cell functioning. Additionally, there is a reduction in autophagy and proteostasis.

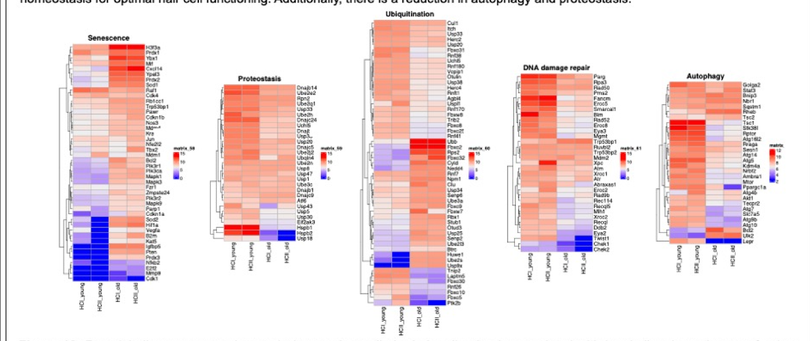


Figure 12: Pseudobulk gene expression analysis reveals vestibular hair cell aging is associated with key hallmarks pathways of aging such as senescence, proteostasis, ubiquitination, DNA damage, and autophagy.

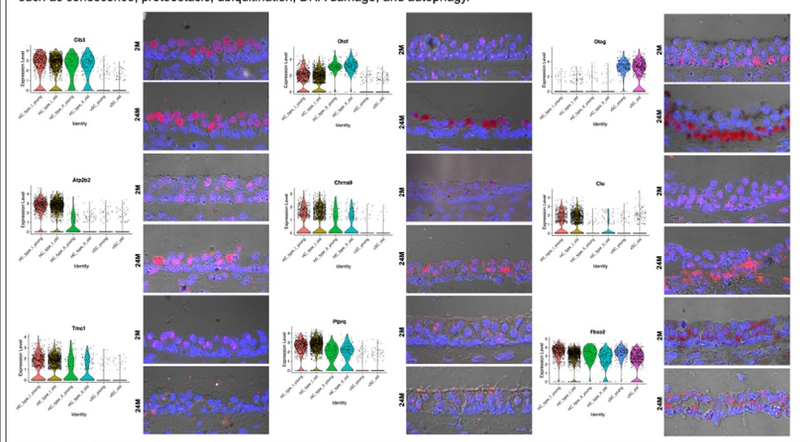


Figure 13: RNAscope *in-situ* hybridization shows age-related alterations in key marker genes related to vestibular hair cells and supporting cell functions.

DISCUSSION & CONCLUSION

- Our functional analysis by VsEP shows age-related vestibular functional decline in our young and aged CBA/J cohorts aligning with the previous findings in the literature.
- Our morphology analysis by H&E staining, scanning electron microscopy, and immunostaining show alterations in cell soma, stereocilia bundle loss, kinocilia, stereocilia bundle degeneration, and bundle fusion with aging.
- Differentially expressed gene analysis of aged hair cells identified mt-Co1, mt-Co3 (oxidative stress), Ush1c, Calm1, Ttc21a, Tuba1a, Evl (bundle maintenance, mechanotransduction, cell soma, kinocilia), Fbxo2, Fbxo32 (ubiquitination), Hsp90aa1, Hsp90ab1 (proteostasis) compared to young hair cells.
- Differentially expressed gene analysis of aged supporting cells identified Epcam, Cntn1, Cxadr, Cldn7 (cell adhesion), Park7, Lamp1 (autophagy), Uba52, Fbxo2 (ubiquitination), Hsp90ab1, Hspa5, Pdia3, Erp29 (protesostasis) compared to young supporting cells.
- GO enrichment analysis further shows a significant downregulation in genes related to stereocilia and kinocilia structure, mechanotransduction, and proteostasis highlighting underlying mechanisms of hair cell aging. Aged supporting cells exhibit a notable decline in ion and fluid homeostasis for optimal hair cell functioning.
- Our pseudobulk gene expression analysis reveals vestibular hair cell aging is associated with global hallmarks pathways of aging such as senescence, proteostasis, ubiquitination, DNA damage, and autophagy.
- scRNA-seq analysis also revealed cell-type specific age-related alterations in HC and SC genes such as Cib3, Atp2b2, Tmc1, Otof, Chrna9, Ptprq, Otog, Clu, Fboxo2 and these changes were validated via RNAscope.
- Overall, our findings will provide a deeper understanding of age-related pathophysiological changes in the vestibule at systemic, cellular and molecular levels shedding light on vestibular aging.

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