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Emerging Mechanisms in the Pathogenesis of Meniere's Disease: Evidence for the Involvement of Ion Homeostatic or Blood-Labyrinthine Barrier Dysfunction in Human Temporal Bones

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Abstract

Hypothesis: Analysis of human temporal bone specimens of patients with Meniere's Disease (MD) may demonstrate altered expression of gene products related to barrier formation and ionic homeostasis within cochlear structures compared to control specimens

Background: MD represents a challenging otologic disorder for investigation. Despite attempts to define the pathogenesis of MD, there remain many gaps in our understanding, including differences in protein expression within the inner ear. Understanding these changes may facilitate the identification of more targeted therapies for MD.

Methods: Human temporal bones from patients with MD (n = 8) and age-matched control patients (n = 8) were processed with immunohistochemistry stains to detect known protein expression related to ionic homeostasis and barrier function in the cochlea, including CLDN11, CLU, KCNJ10, and SLC12A2. Immunofluorescence intensity analysis was performed to quantify protein expression in the stria vascularis (SV), organ of Corti (OoC), and spiral ganglion (SGN).

Results: Expression of KCNJ10 was significantly reduced in all cochlear regions, including the SV (9.23 v. 17.52, p=0.011), OC (14.93 v. 29.16, p=0.014), and SGN (7.69 v. 18.85, p=0.0048) in human temporal bone specimens from patients with MD compared to control, respectively. CLDN11 (7.40 v. 10.88, p=0.049) and CLU (7.80 v. 17.51, p= 0.0051) expression was significantly reduced in the SGN.

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Conflicts of Interest:
None.

Conclusion: The results of this study support that there may be differences in the expression of proteins related to ionic homeostasis and barrier function within the cochlea, potentially supporting the role of targeted therapies to treat MD.

Keywords

Meniere's Disease; human temporal bone; KCNJ10; CLU; CLDN11; SLC12A2; histopathology

Introduction:

Meniere's Disease (MD) represents a debilitating otologic disorder that is characterized by fluctuating sensorineural hearing loss (SNHL), episodic vertigo, tinnitus, and aural fullness. Due to the transient nature of the symptoms, MD represents a challenging otologic disorder for investigation. Prior studies have highlighted a few phenotypic findings within the inner ear, including endolymphatic hydrops (EH) and atrophy of many cochlear structures[1–6]. Furthermore, there is recent evidence supporting differential expression of target genes involved in ionic and fluid homeostasis within the cochlea, including circumstantial evidence for involvement of the stria vascularis, a major contributor to ionic homeostasis[7]. Currently, an animal model that replicates Meniere's disease in its entirety does not exist. Despite attempts to define the pathogenesis of MD, there remain many gaps in our understanding, including underlying etiology (genetic or otherwise), cochlear cell types responsible for the underlying pathophysiology, as well as differences in critical cell type-specific protein expression within the human inner ear. This study reports evidence of differential expression of proteins related to barrier formation and ionic homeostasis within cochlear structures of human temporal bones of patients with MD compared to age-matched control specimens. These results support the need of the future investigation to design targeted therapies for MD.

Methods:

Human temporal bones from patients with diagnosis of MD (n = 8) and age-matched control patients (n = 8) were obtained from the NIDCD national temporal bone laboratory (Table 1). The studies involving human participants were reviewed and approved by the University of California at Los Angeles Institutional Review Board (IRB protocol #10–001449 and # 22–001587). Appropriate informed consent for inclusion in the study was obtained from each temporal bone donor.

Temporal bone specimens were processed for immunohistochemical analysis per previously published methods[8,9]. Detailed procedures of temporal bone collection, fixation, decalcification and celloidin embedding were described by Merchant and Nadol[10].

Celloidin removal and antigen retrieval: The methodology for celloidin removal and antigen retrieval has been described in detail (Lopez et al., 2016). In brief, celloidin sections were immersed in sodium-ethoxide (saturated solution) diluted in 100% ethyl alcohol (1:3, 60 min), 100% ethanol (2 times, 5 min), and distilled water (3×10 min). Sections were immersed in heated antigen retrieval solution - 100°C - diluted 1:500 in double-distilled water (Vector antigen unmasking Acidic solution, Vector Labs, Burlingame, CA). Sections

were allowed to cool for 30 min, washed with phosphate-buffered saline (3x 5 min, PBS) and immediately incubated for 8 minutes in a diluted trypsin solution (1:3, Abcam Trypsin Kit) and washed 4×10 minutes in PBS before immunofluorescence.

Immunofluorescence (IF): Sections were incubated for 2 hours with a blocking solution containing 1% bovine serum albumin (BSA) fraction-V (Sigma, St. Louis, MO) and 0.5% Triton X-100 (Sigma) in PBS. Followed by the incubation with the primary antibodies diluted in PBS for hours at 4 °C in a humid chamber. After the 5 days of incubation, primary antibodies were removed by 4 × 15 minutes PBS washing step.

Primary antibodies were selected based on known cell type-specificity in the stria vascularis based on published single-cell and single-nucleus transcriptome data from the adult mouse stria vascularis[11,12]. The following primary antibodies were used: rabbit anti-KCNJ10 (RRID:AB_2040120, Alomone Labs, APC-035, polyclonal, dilution 1:200), rabbit anti-CLDN11 (RRID: AB_2533259, Invitrogen, 364500, polyclonal, dilution 1:200), goat anti-SLC12A2 (RRID:AB_2188633, Santa Cruz Biotechnology, sc-21 545, polyclonal, dilution 1:200), goat anti-CLU (RRID:AB_2083314, R and D Systems, polyclonal, dilution 1:200).

The sections were incubated with secondary antibodies: donkey anti rabbit (Alexa 555, 1:1000 in PBS), and donkey anti goat (Alexa 647, 1:1000 in PBS) for 3 hrs. The tissue sections were then washed with PBS (3 × 15 minutes) and coverslip with aqua soluble mounting media containing DAPI to visualize cell nuclei (Vectashield, Vector).

Antibodies were used to label at least 5 human specimens each from different individuals. Imaging was performed using a Zeiss LSM810 confocal microscope at 40X magnification for each turn of the cochlea for the stria vascularis (SV), organ of Corti (OC), and spiral ganglion neurons (SGN). After conversion of images to grayscale, fluorescence intensity quantification was performed in ImageJ by calculating the fluorescence intensity of the outlined region of the SV as previously described[9,13,14]. Fluorescence intensity was normalized by comparing SV fluorescence intensity to a corresponding region in the scala media. Negative controls consisted of secondary antibody only and unstained human sections to assess for background staining and autofluorescence, respectively. Negative controls exhibited minimal staining or autofluorescence (data not shown). Analysis of immunofluorescence intensity was performed, and comparisons made between cochlea from patients with and without MD using an unpaired t-test with p-values less than 0.05 as the threshold for significance.

Results:

Immunofluorescence intensity analysis was performed to assess the degrees of expression of proteins implicated in ion homeostasis and blood labyrinth barrier regulation that had previously been localized to cochlear structures, including CLDN11, CLU, KCNJ10, and SLC12A2. The findings are summarized in Table 2.

There were directionally, non-significant reductions in expression of CLDN11 in the SV (17.86 v. 7.946, p=0.0575, Figure 1A–C) and OC (10.28 v. 6.164, p=0.0501, Figure 1D–F), with significantly decreased expression within the SGN (10.88 v. 7.397, p=0.0497, Figure

1G–I) in human temporal bone specimens from control patients compared to MD patients, respectively. There were no significant differences in expression of CLU in the SV (17.54 v. 11.77, p=0.1465, Figure 2A–C) or OC (17.53 v. 11.67, p=0.2782, Figure 2D–F), however, there were significant reductions of CLU expression within the SGN (16.05 v. 7.669, p= 0.0183, Figure 2G–I) in control compared to MD specimens, respectively. KCNJ10 levels were significantly reduced in all regions, including the SV (16.75 v. 8.815, p=0.0151, Figure 3A–C), OC (27.55 v. 14.50, p=0.0256, Figure 3D–F), and SGN (18.31 v. 6.558 v., p=0.0015, Figure 3G–I). There were no significant differences between control and MD specimens in expression of SLC12A2 for the SV (13.26 v. 10.36, p=0.2278, Figure 4A–C), (7.416 v. 8.102, p=0.8019, Figure 4D–F), or SGN (18.57 v. 10.54, p=0.2013, Figure 4G–I).

These results highlight the first evidence of decreased differential expression of proteins associated with ionic homeostasis (KCNJ10) in all tested cell-types within the cochlea in patient, as well as decreased expression of CLDN11 and CLU within the SGN.

Discussion:

Meniere's disease (MD) represents an otologic disorder that presents many challenges to pathophysiologic investigation due to the fluctuating and heterogeneous presentation of symptoms and relative lack of animal models that completely recapitulate the disease. Recent cell type-specific data regarding the presence of proteins associated with ionic and fluid homeostasis in the inner ear have provided an opportunity to compare differences in the expression of these proteins in patients with MD. By demonstrating differential expression of proteins implicated in ion homeostasis and blood labyrinth barrier formation, the current study provides the first evidence for ionic homeostatic dysfunction in the cochlea in these patients.

1. Overview of Meniere's Disease (MD)

Since first described by Prosper Meniere in 1861 as a disorder of fluctuating sensorineural hearing loss (SNHL), tinnitus, aural fullness, and vertigo, MD has remained a challenging otologic disorder for both diagnosis and management[15]. In 2015, the Classification Committee of the Barany Society streamlined the diagnostic criteria for MD to "definite MD" and "probable MD," with definite MD including documented audiometric criteria of low to medium-frequency SNHL in addition to symptomatology[16].

Although the etiology of MD remains incompletely understood, there have been studies supporting the role of various genetic and epigenetic factors in the pathophysiology of MD[17]. Although most cases are believed to be sporadic, there is evidence supporting a subset of familial MD (5–15%)[18–20] in addition to various comorbidity and abnormal immune response mechanisms[21,22]. MD may represent a spectrum of heterogenous disorders with a common, variable phenotype that contributes to the relative complexity in diagnosis and dearth of effective therapeutic treatments.

Human temporal bone histopathology has consistently demonstrated the presence of endolymphatic hydrops (EH), a dilation of the endolymph-containing compartments of the cochlea and labyrinth, in affected and unaffected ears of patients with MD, suggesting the

possibility that EH represents an epiphenomenon rather than a causal factor for MD[1,2,23]. In addition, other inner ear structures have been implicated in humans including hair cells, spiral ganglion neurons (SGN), supporting cells, stria vascularis (SV), saccule, utricle, and endolymphatic sac[2–6]. Furthermore, atrophy of the SV, as well as a decrease in the mean number of SV blood vessels have been previously reported[3–5]. Thus, these observations suggest that fluid and ion homeostasis, as well as blood-labyrinth barrier function, may underlie MD disease pathophysiology.

2. Ionic and Fluid Homeostasis of the Inner Ear

The endolymph is a fluid within the membranous labyrinth of the inner ear that possesses a unique chemical composition that is relatively high in potassium compared to the perilymph and other bodily fluids. The SV is a specialized, non-sensory epithelium within the lateral wall of the cochlea that is one of the cell types that tightly regulates ionic homeostasis of the fluid compartments within the cochlea. The SV contributes to the maintenance of the high potassium concentration in the endolymph by facilitating potassium recycling from the perilymph to the endolymph, contributing to the endocochlear potential (EP), which enables optimal hair cell mechanotransduction[24–27].

It is believed that perilymphatic potassium is primarily transported through the sodium-potassium adenosine triphosphatase (Na/K-ATPase, ATP1A1) and sodium-potassium-chloride (NKCC1, also known as SLC12A2) transporters of fibrocytes (type II and IV) within the spiral ligament[28–30]. These fibrocytes are closely associated with the basal cells and intermediate cells of the SV through gap junctions, such as connexin-26 (CX26), facilitating passive transport of potassium[31–33]. Potassium migrates from the intermediate cells into the intrastrial space through inwardly rectifying potassium channels, such as KCNJ10 (also known as Kir4.1)[34,35]. In order to keep the intrastrial potassium low, marginal cells efficiently transport potassium from the intrastrial space across their basolateral membranes utilizing Na/K-ATPase transporter (encoded by *ATP1A1* and *ATP1B2*) and NKCC1 cotransporter or SLC12A2 protein (encoded by *SLC12A2*)[36,37]. Potassium is then secreted from the apical membranes of the marginal cells into the relatively potassium-rich endolymph through voltage-gated potassium channels, such as KCNQ1 and KCNE1[38,39]. The maintenance of the relatively privileged electrochemical environment of the cochlear endolymph is further supported by paracellular tight junctions, such as claudin-11 (CLDN11), among others, which tightly regulate the passage of ions[40]. Disruption of any of these processes of potassium recycling and generation of the EP have been shown to result in hearing loss[41]. Following membrane depolarization as potassium enters the cells, voltage-gated calcium channels open to transmit a neural signal to the auditory nerve via the spiral ganglion neurons (SGN)[42]. While the contribution of the SGN to potassium recycling within the inner ear remains poorly understood, studies have demonstrated the presence of voltage-gated potassium channels, including Kv3 (KCNC1) and Kv1 (KCNA1), and potassium leak channels (KCNK) within SGN cells, that contribute to the proper transduction of electrical signals along the cochlear afferent neural pathway[43–46].

3. Review of Targeted Proteins Associated with Ion and Fluid Homeostasis of the Inner Ear

Although there are numerous proteins involved in fluid and ion homeostasis of the inner ear, this study performed analysis of a few select proteins that demonstrate cell-specific expression with commercially available antibodies. There are no previous studies directly implicating differential expression of these proteins in the pathophysiology of MD. Here we will briefly review the expression and current understanding of the function of these selected proteins in relation to hearing disorders.

a. Claudin-11 (CLDN11)—Tight junctions are a family of proteins that contribute to the integrity of cellular barrier formation, regulating paracellular permeability and the maintenance of distinct fluid electrochemical compositions[47]. Tight junctions may be found throughout many mammalian tissues, with claudins and occludins, among others, forming the cellular binding of membrane proteins within the inner ear. Approximately 24 claudins have been identified in humans, with at least 10 subtypes identified within inner ear tissues[48]. Claudin-1,2,3,9,10,12,14, and 18 have been demonstrated in multiple tissue types including organ of Corti, Reissner's membrane, spiral limbus, and marginal cells of SV; claudin-11 (CLDN11) represents a unique tight junction protein that has been localized to basal cells of the SV[48]. CLDN11 has been shown to be critical for the maintenance of EP and hearing function[49]. CLDN11 knockout mice have demonstrated profound hearing loss associated with reduced EP[50]. Claudin proteins have been implicated in various deafness disorders, including claudin-14 in human deafness DFNB29[51].

b. Clusterin (CLU)—Clusterin (CLU) is an extracellular chaperone glycoprotein that has been identified within many tissues in the body, including the inner ear[52]. While the specific role of CLU remains incompletely understood, it is believed to play a role in the oxidative stress response and proteostasis, as well as blood-brain barrier formation[53]. Previous RNA-seq data demonstrated CLU expression in hair cells and supporting cells within the inner ear during development[54,55] and in outer hair cells, supporting cells (Deiters and pillar cells), cells of the outer sulcus and SV basal cells in the adult mouse[56,57]. Although there have been no prior human tissue studies with regards to CLU expression in hearing loss, there exists a knockout mouse model supporting a potential protective role of CLU against age-related hearing loss and aminoglycoside ototoxicity[58].

c. Potassium Inwardly Rectifying Channel (KCNJ10)—The inwardly rectifying potassium channel KCNJ10, also known as Kir4.1, is expressed in various tissues including the inner ear, eye, brain, erythrocytes, endothelial cells, and renal tubules[59]. Within the inner ear, KCNJ10 expression has been demonstrated within cochlear lateral wall, specifically the intermediate cells of the SV, SGN cells, and supporting cells in the organ of Corti[35,60,61]. These inward rectifying potassium channels exhibit differential conductance for polarization status of cells, with higher conductance during cellular hyperpolarization and lower conductance during action potential depolarization, allowing electrochemical current to flow into the cell. These channels have been shown to facilitate movement of potassium from intermediate cells of the SV to the intrastrial space[61]. KCNJ10 channel has been hypothesized to regulate the cellular membrane potential via potassium (K+) diffusion

through Barium (Ba2+)-sensitive channels within the intermediate cells[35,61,62]. Disruption of the KCNJ10 channel has been shown to result in reduction in EP and deafness in animal models[34,63].

d. Sodium-Potassium-Chloride Cotransporter (NKCC1/SLC12A2)—The NKCC protein family aids in electroneutral transport of sodium, potassium, and chloride across plasma membranes of cells. NKCC1, encoded by *SLC12A2*, is found in many cells of the body and functions for osmotic regulation through the electroneutral flux of ions into cells. NKCC1 has been previously shown to be expressed in the basolateral membrane of SV marginal cells, fibrocytes of the spiral ligament (SL) and spiral limbus, and satellite glial cells surrounding spiral ganglion neurons (SGN)[28,29,64,65]. Examination of single-cell RNA-Seq data from adult mouse spiral ganglion neurons and satellite glial cells on gEAR[46,66,67] demonstrate expression of SLC12A2 in both SGNs and satellite glial cells (data not shown). Animal models have demonstrated reduction in NKCC expression results in elevated hearing thresholds in knockout mice[68].

4. Implications of Ion and Fluid Homeostasis in Meniere's Disease (MD)

Prior studies have demonstrated that disruption of the function of these ion channels or junction proteins may result in SNHL[69]. Given the prior evidence of EH and structural changes within the cell types of the cochlea in human temporal bone studies of patients with MD, there have been attempts to identify potential genetic and transcriptomic changes within the inner ear of these patients. A recent paper by Gu et al. (2021) performed a systematic literature review, highlighting 832 genes implicated in MD across 77 studies[7]. These reports detail the identification of target proteins and their location within the inner ear.

The current study demonstrates the first report of differential expression of proteins associated with ion and fluid homeostasis within human temporal bones of patients with MD compared to control patients. The results demonstrate that KCNJ10 protein expression was noted to be significantly reduced in all investigated cell-types, including the SV (16.75 v. 8.815, p=0.0151), OC (27.55 v. 14.50, p=0.0256), and SGN (18.31 v. 6.558, p=0.0015) in specimens from control patients compared to MD patients, respectively. This represents an important finding as KCNJ10 remains a critical modulator of potassium movement within the inner ear, implicating a potential association with dysregulation of ion and fluid homeostasis that may contribute to the pathogenesis of MD. This supports prior reports by Gallego-Martinez et al. (2019) demonstrating that KCNJ10 has been associated with missense variants in a large case series of sporadic MD cases[22].

Previous studies demonstrating reductions in ATP1A1 expression in the stria vascularis and the saccule of patients with Meniere's disease lend credence to the assertion that dysregulation of ion and fluid homeostasis may underlie MD[70,71]. Additionally, CLDN11 represents a tight junction regulator of ion and fluid movement in the inner ear and expression was shown to be significantly reduced in the SGN (17.86 v. 7.946, p=0.0497) with reduced but not significant reductions in SV (17.86 v. 7.946, p=0.0575) and OC (10.28 v. 6.164, p=0.0501). Furthermore, CLU was found to be significantly decreased within

the SGN of control patients compared to the MD cohort, respectively (16.05 v. 7.669, p= 0.0183). While there were no significant differences between expression of SLC12A2 between MD and control specimens in all cell types, the potential role of this protein in the pathogenesis of MD remains poorly understood. While the SV has been implicated as the primary site of EP generation, there are other tissues, such as the OC and SGN, that may also play a role in ion and fluid homeostasis within the inner ear. In the present study, the most pronounced reductions in the expression of KCNJ10, CLDN11, and CLU in human temporal bone specimens from MD patients were found in the SGN. As previously discussed, the role of the SGN in ion and fluid homeostasis remain incompletely understood, highlighting the need for further studies on these inner ear tissues.

There remain many limitations to this study, including the limited number of proteins and tissues assessed. While the proteins were selected due to commercially available antibodies with demonstrated cell-specific expression allowing greater feasibility of quantification and comparison, there are numerous other proteins and hormonal influences that may contribute to ion and fluid homeostasis in the inner ear that may play a role in the pathogenesis of MD. Another limitation is that structural and immunohistochemical changes observed in diseases like Meniere's disease in human temporal bone histopathology represent the end stages of these diseases and may not represent the active disease process. Therefore, the current study is unable to distinguish whether the differential protein expression represents the causative etiology of the disease process. Nonetheless, recent mouse model evidence of SV dysfunction resulting in subsequent delayed SGN dysfunction[72] is supportive of our observations of changes in temporal bones from patients with Meniere's disease.

Conclusion:

This study presents the first report of differential expression of previously identified SV cell-type specific proteins associated with fluid and ionic homeostasis and blood-labyrinth barrier function[7,11], in human temporal bones of patients with MD. While the SV serves as a window into fluid and ion homeostasis in the cochlea, this study points to the importance of considering ion homeostasis function not just in the SV but also in other areas of the cochlea including the OC and SGN regions of the cochlea. We demonstrate decreased expression of KCNJ10 in all investigated regions (SV, OC, and SGN) and decreased expression of CLDN11 and CLU in SGN cells in human temporal bone specimens of MD patients. This study adds to the existing literature on differential expression of proteins associated with fluid and ion homeostasis, by characterizing the differential expression of known SV cell type-specific proteins within the inner ear of patients with MD. Further research is needed to better understand the implications of dysfunctional fluid and ion homeostasis in the pathogenesis of MD and may assist in the development of future targeted therapeutics.

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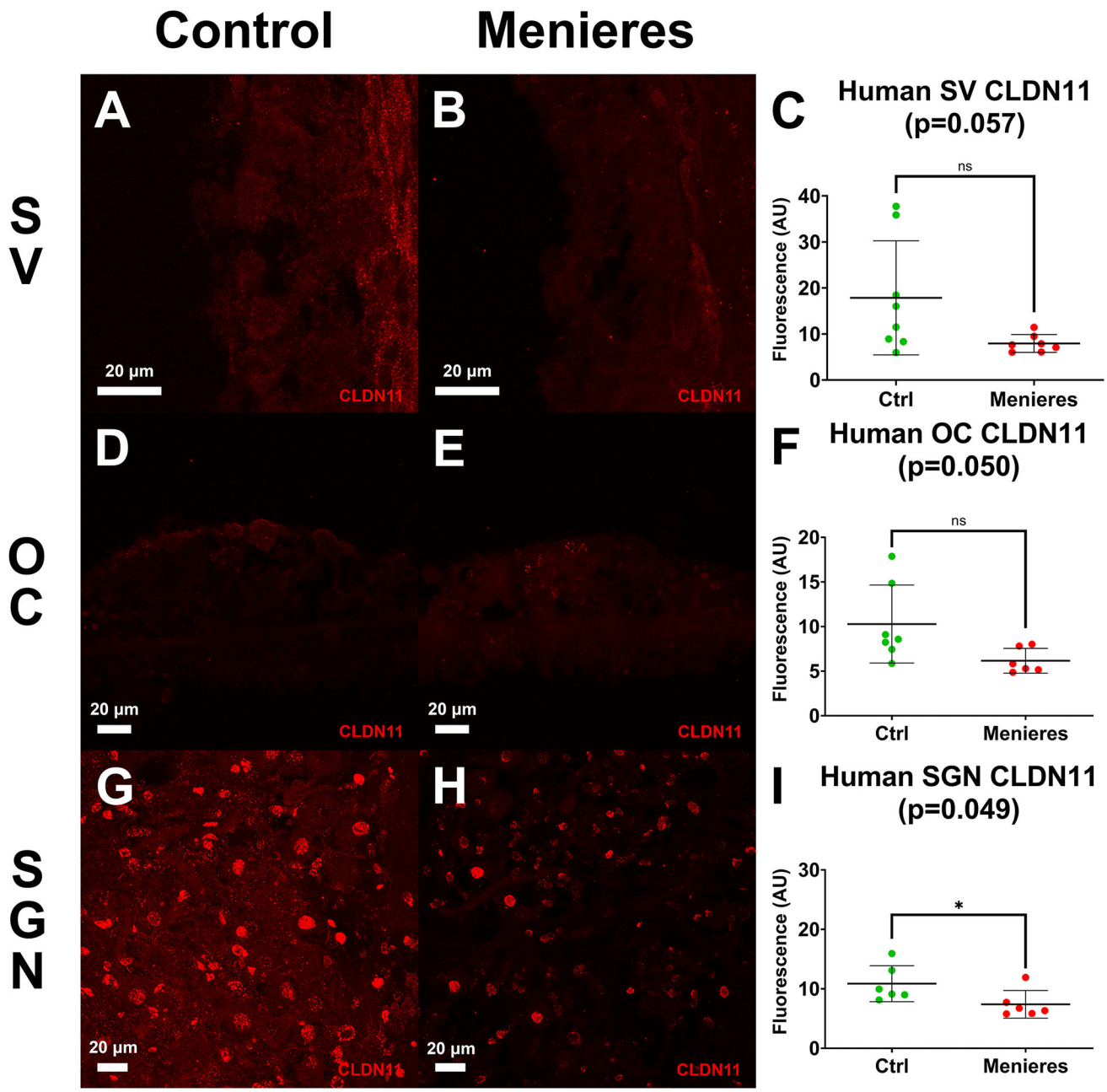

Figure 1. Average fluorescence intensity of CLDN11 by cochlear region.

(A-C), Representative CLDN11 immunolabeling of the stria vascularis in control (A) and Meniere's disease (B) patients with quantitative analysis of the immunofluorescence intensity of CLDN11 antibody labeling shown in panel C. Note the expected labeling of the basal cell layer of the stria vascularis (far right of panels A and B), which is the third layer of SV cells which lie the furthest away from the scala media (left side of image in panels A and B). **(D-F)**, Representative CLDN11 immunolabeling of the organ of Corti in control (D) and Meniere's disease (E) patients with quantitative analysis of the immunofluorescence intensity of CLDN11 antibody labeling shown in panel F. Immunolabeling with CLDN11 antibody is absent as expected in the organ of Corti region in both control and Meniere's

disease patients. **(G-I)**, Representative CLDN11 immunolabeling of the spiral ganglion region in control (G) and Meniere's disease (H) patients with quantitative analysis of the immunofluorescence intensity of CLDN11 antibody labeling shown in panel I.

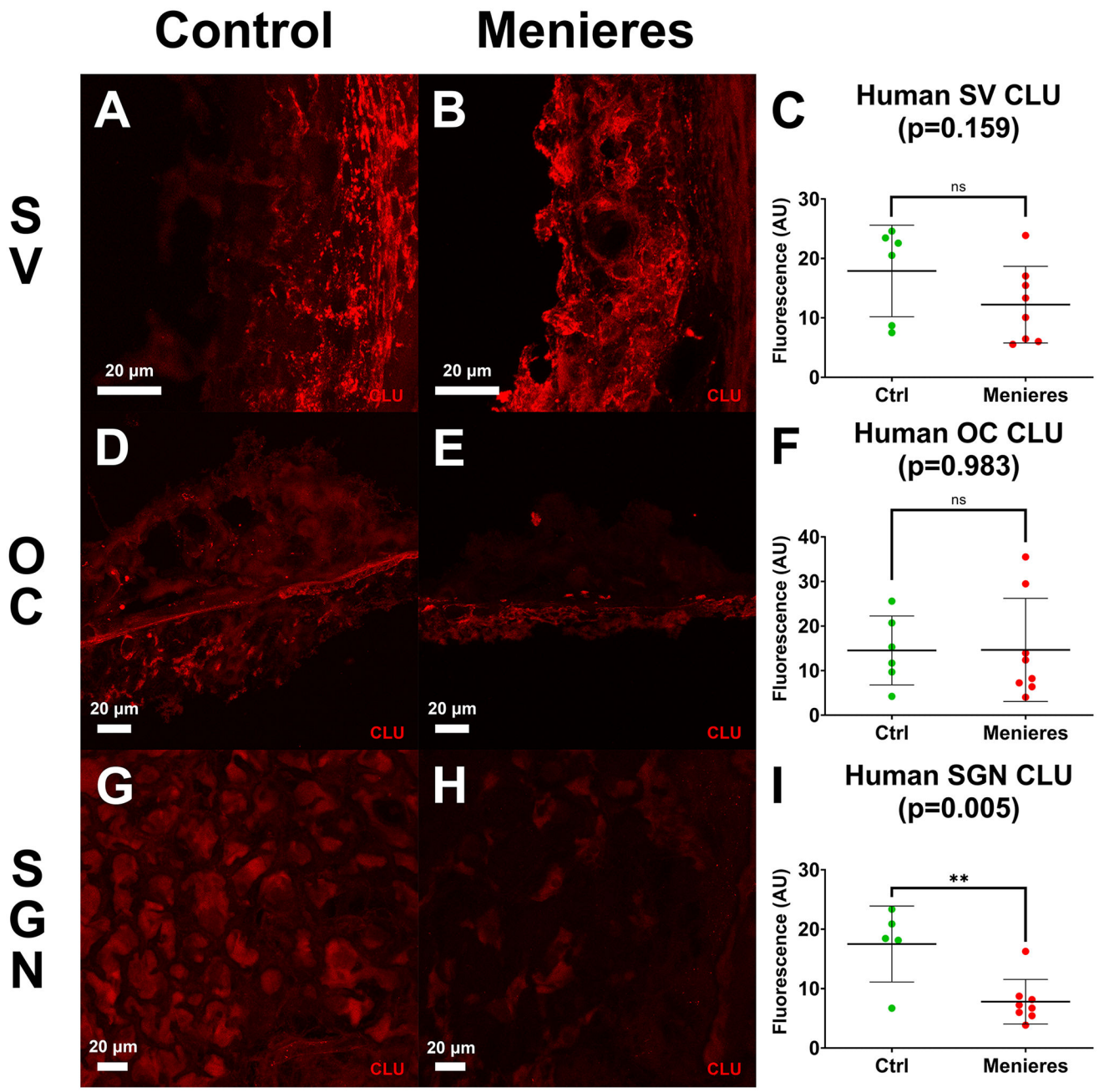

Figure 2. Average fluorescence intensity of CLU by cochlear region.

(A-C), Representative CLU immunolabeling of the stria vascularis in control (A) and Meniere's disease (B) patients with quantitative analysis of the immunofluorescence intensity of CLU antibody labeling shown in panel C. **(D-F)**, Representative CLU immunolabeling of the organ of Corti in control (D) and Meniere's disease (E) patients with quantitative analysis of the immunofluorescence intensity of CLU antibody labeling shown in panel F. **(G-I)**, Representative CLU immunolabeling of the spiral ganglion region in control (G) and Meniere's disease (H) patients with quantitative analysis of the immunofluorescence intensity of CLU antibody labeling shown in panel I.

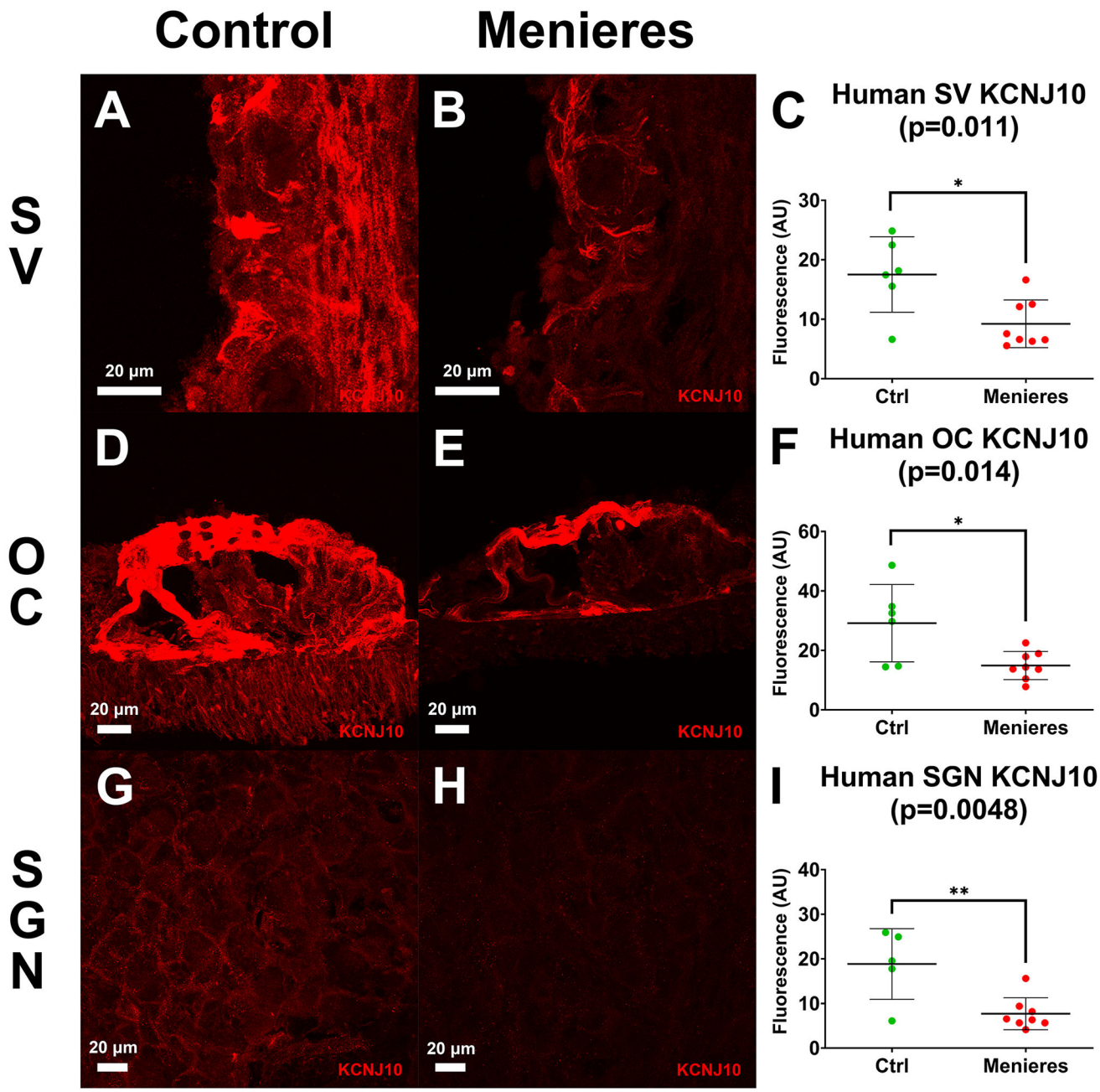

Figure 3. Average fluorescence intensity of KCNJ10 by cochlear region.

(A-C), Representative KCNJ10 immunolabeling of the stria vascularis in control (A) and Meniere's disease (B) patients with quantitative analysis of the immunofluorescence intensity of KCNJ10 antibody labeling shown in panel C. Note that KCNJ10 immunolabeling is seem predominantly within the stria vascularis which is consistent with its known expression in intermediate cells of the stria vascularis. **(D-F)**, Representative KCNJ10 immunolabeling of the organ of Corti in control (D) and Meniere's disease (E) patients with quantitative analysis of the immunofluorescence intensity of KCNJ10 antibody labeling shown in panel F. Note KCNJ10 immunolabeling is seen in supporting cells, notably the pillar cells and in the reticular lamina over the tops of the outer hair cells which

is formed by the phalangeal processes of the Deiters cells. **(G-I)**, Representative KCNJ10 immunolabeling of the spiral ganglion region in control (G) and Meniere's disease (H) patients with quantitative analysis of the immunofluorescence intensity of KCNJ10 antibody labeling shown in panel I.

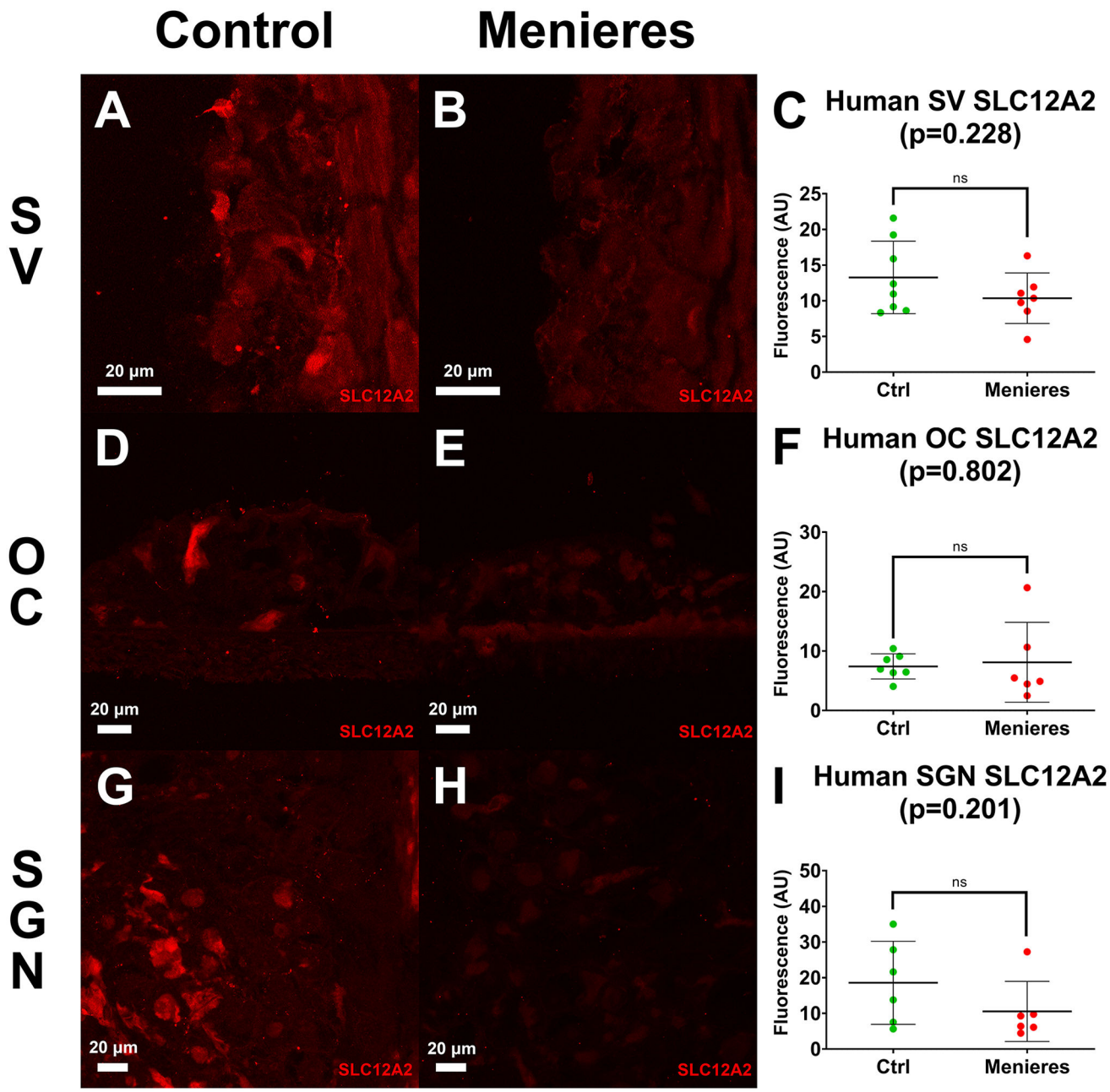

Figure 4. Average fluorescence intensity of SLC12A2 by cochlear region.

(A-C), Representative SLC12A2 immunolabeling of the stria vascularis in control (A) and Meniere's disease (B) patients with quantitative analysis of the immunofluorescence intensity of SLC12A2 antibody labeling shown in panel C. **(D-F)**, Representative SLC12A2 immunolabeling of the organ of Corti in control (D) and Meniere's disease (E) patients with quantitative analysis of the immunofluorescence intensity of SLC12A2 antibody labeling shown in panel F. **(G-I)**, Representative SLC12A2 immunolabeling of the spiral ganglion region in control (G) and Meniere's disease (H) patients with quantitative analysis of the immunofluorescence intensity of SLC12A2 antibody labeling shown in panel I.

Table 1.

Demographic information for temporal bones included in study.

Specimen	Age	Gender	Diagnosis
1	74	M	MD
2	52	M	MD
3	55	F	MD
4	86	M	MD
5	56	M	MD
6	92	F	MD
7	69	F	MD
8	79	M	MD
9	79	M	Normal
10	71	M	Normal
11	74	F	Normal
12	32	M	Normal
13	59	F	Normal
14	30	M	Normal
15	20	M	Normal
16	10	M	Normal

Abbreviation. F: female, M: male. Age in years. MD: Meniere's disease.

Table 2.

Comparison of average fluorescence intensity values between control and Meniere's Disease human temporal bone specimens for each protein stratified by region of cochlea, including stria vascularis (SV), organ of Corti (OC), and spiral ganglion neuron (SGN).

Stria Vascularis (SV)			
	Control	**Meniere's Disease**	**p-value**
CLDN11	17.86	7.946	0.0575
	(n=8)	(n=7)	
CLU	17.89	12.23	0.1599
	(n=6)	(n=8)	
KCNJ10	17.52	9.227	**0.011** *
	(n=6)	(n=8)	
SLC12A2	13.26	10.36	0.2278
	(n=8)	(n=7)	
Organ of Corti (OC)			
	Control	**Meniere's Disease**	**p-value**
CLDN11	10.28	6.164	0.0501
	(n=7)	(n=8)	
CLU	14.53	14.65	0.9829
	(n=6)	(n=8)	
KCNJ10	29.16	14.93	**0.0138** *
	(n=6)	(n=8)	
SLC12A2	7.416	8.102	0.8019
	(n=7)	(n=6)	
Spiral Ganglion Neuron (SGN)			
	Control	**Meniere's Disease**	**p-value**
CLDN11	10.88	7.397	**0.0497** *
	(n=6)	(n=6)	
CLU	17.51	7.802	**0.0051** *
	(n=5)	(n=8)	
KCNJ10	18.85	7.69	**0.0048** *
	(n=5)	(n=8)	
SLC12A2	18.57	10.54	0.2013
	(n=6)	(n=6)	

* Statistical significance indicated if $p<0.05$ by unpaired t-test