Ménière’s Disease Pathophysiology: Endolymphatic Sac Immunohistochemical Study of Aquaporin-2, V2R Vasopressin Receptor, NKCC2, and TRPV4

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Abstract

Objectives. Endolymphatic sac (ELS) pathophysiology in Ménière’s disease (MD) remains poorly understood. We identified from the literature a group of proteins expressed on the ELS and involved in endolymph volume regulation: aquaporin-2 (AQP2), vasopressin receptor V2R, sodium potassium chloride cotransporter 2 (NKCC2), and transient receptor potential cation channel V4 (TRPV4). Our objective was to determine whether their ELS expression was altered in MD, to better understand the pathophysiology of endolymphatic hydrops.

Study Design. Prospective case-control study.

Setting. Tertiary care center.

Subjects. Twenty-four patients with definite MD undergoing endolymphatic duct blockage surgery were recruited, as well as 23 controls with no history of MD undergoing surgery for vestibular schwannoma (VS).

Methods. ELS biopsies and blood samples for plasma arginine vasopressin (AVP) were obtained. Immunohistochemistry for AQP2, V2R, NKCC2, and TRPV4 was performed. Slides were scanned digitally for highly sensitive pixel density analysis by specialized software (VIS; Visiopharm).

Results. Global scores generated by the software represent total and relative protein expression density of 3 staining intensity levels, exclusively on ELS epithelium. AQP2 expression density was significantly elevated in MD compared to VS (P = .003). There was no significant difference in plasma AVP, V2R, NKCC2, and TRPV4 expression.

Conclusion. This original study evaluates simultaneous in situ expression of AQP2, V2R, NKCC2, and TRPV4 on the human ELS in MD, with a control group. Our results show only AQP2 upregulation on the ELS of patients with MD. We suggest a constitutively increased expression of AQP2 in MD, independent of its regulatory axis (AVP-V2R). Acquired regulator sequence mutations could support this model.

Keywords

Ménière’s disease, vestibular schwannoma, endolymphatic sac, duct blockage, aquaporin-2, V2 receptor, vasopressin, NKCC2, TRPV4

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Ménière’s disease (MD) is characterized by episodic vertigo, fluctuating sensorineural hearing loss, and aural symptoms. The underlying pathology is not well understood with many proposed etiologies.1-8 Refractory MD has been challenging for otologists as there is no solid evidence for the efficacy of most therapeutic options.9 We have described a novel technique that controls symptoms with considerable success, supported by 5 years of experience: endolymphatic duct blockage surgery. It consists of a modified endolymphatic sac decompression followed by a crucial therapeutic step: blocking the endolymphatic duct with 2 titanium clips.10

The endolymphatic (EL) compartment extends from the scala media to the endolymphatic sac (ELS). Although still debated, experiments suggest ELS is a site of fluid resorption.11 Endolymphatic hydrops (ELH) is the pathologic hallmark of MD and has been researched extensively, but its contribution to symptoms remains unclear; magnetic resonance (MR) imaging studies suggest that ELH may develop in patients well before symptoms manifest.12-17

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Aquaporins (AQPs) are transmembrane proteins that facilitate water transport. Their importance for auditory function is supported by experimental models: AQP knock-out mice are born with profound hearing loss. The ELS is often compared to the kidney due to histological similarities (cell types and their disposition/function). Twenty percent of water reabsorbed by the renal collecting duct is regulated by vasopressin (AVP): V2R receptor stimulation results in aquaporin-2 (AQP2) translocation to the luminal membrane via cAMP signaling. In the ELS, the luminal (apical) epithelium is in contact with EL while the basolateral membrane is in contact with connective tissue and blood vessels. AQP2 expression has been confirmed experimentally, mostly stored in endosomal vesicles with the majority of cellular stores confined to endosomal vesicles. Furthermore, ELH is induced by cAMP agonists and reduced by cAMP inhibitors, suggesting an AVP-V2R-AQP2 involvement in the ELS similar to the renal model. Maekawa et al reported elevated AQP2 and V2R messenger RNA (mRNA) and protein concentration in homogenized ELS tissue of patients with MD compared to vestibular schwannoma controls. Furthermore, they documented AQP2 translocation to the basolateral membrane following AVP stimulation. In response to AVP, luminal AQP2 is endocytosed to form new vesicles, reducing water influx from the sac lumen. Simultaneously, multiple vesicles fuse with the basolateral membrane (in contact with blood vessels), and water flows between the cell and blood until osmotic equilibrium is reached. The result is a restriction of water movement from the sac lumen across the epithelium.

NKCC2 (sodium potassium chloride co-transporter) pumps ions from the extracellular compartment into the cell. In the ELS, the equilibrium between K⁺-rich EL in the cochlea and Na⁺-rich EL in the ELS can only be achieved by active transport. NKCC2 expression has been confirmed on the luminal ELS membrane in both humans and animals. TRPV4 is a calcium-dependent calcium channel activated by hypotonicity, mechanical cell swelling, and heat. Expression of TRPV4 was confirmed in guinea pig and human ELS. The exact role of TRPV4 in cellular fluid balance is poorly understood but is of interest in MD: one group confirmed luminal membrane TRPV4 expression in addition to its involvement in osmoregulation.

The objective of our ELS study is to determine if AQP2, V2R, NKCC2, and TRPV4 in situ protein expression is altered in MD. This approach has the advantage of determining protein concentration at the functional site rather than any ELS structure. Confirming elevated levels of plasma vasopressin (pAVP) in MD is our secondary objective.

**Materials and Methods**

**Study Design and Patient Population**

This is a cross-sectional, single-physician, case-control study conducted from 2014 to 2017 at our tertiary care center, following University of Montreal Hospital Center’s institutional review board approval. Patients with a clinical diagnosis of definite MD according to the 1995 American Academy of Otolaryngology—Head and Neck Surgery (AAO-HNS) criteria opted for endolymphatic duct blockage surgery (EDB). Eligible controls had a diagnosis of vestibular schwannoma (VS) confirmed by magnetic resonance imaging and presenting for total excision by translabyrinthine approach. Informed consent was obtained for all cases. A total of 27 patients with MD and 23 with VS were enrolled.

**Inclusion criteria:**

1. Failure of medical therapy (diuretics, betahistine, and dietary restrictions) for at least 6 months
2. At least 6 episodes of vertigo consistent with MD in the past 6 months and at least 1 episode in the last month before surgery
3. Controls only: no history of mastoid surgery; intact endolymphatic sac confirmed in the operating room

**Exclusion criteria:**

1. Age under 18 years
2. History of ear surgery
3. Documented otologic (different from MD), cardiac, or renal conditions
4. Documented syndrome or inner ear malformation
5. Controls only: no history of MD

**Endolympathic Sac Tissue Biopsy and Blood Sampling**

During EDB surgery for MD cases, biopsies were obtained from the lateral portion of the ELS main body after clipping of the EL duct. For VS controls, the ELS lateral portion was totally obtained. Tissue samples were transported to the laboratory in a histological fixative (Tissufix T-20; Chaptec, Montreal QC, Canada). Blood samples for plasma vasopressin were obtained preoperatively from all participants. All tissue samples were successfully collected and used for analysis. Although the ELS biopsies were smaller in the MD group, there was more than enough tissue for our experiment. No missing data were noted.

**Histology, Antibodies, and Immunohistochemistry**

ELS biopsies were set in fixative for 24 hours at room temperature (RT). ELS tissues were then embedded with paraffin and cut with a microtome (4 microns thickness). Control samples were sectioned at the most lateral part of the ELS. Five adjacent sections per sample were mounted on slides and stained, of which 4 were reserved for immunohistochemistry (IHC) (stained with hematoxylin and eosin), and 1 was stained with hematoxylin, phloxine, and Safran (HPS). This protocol stains collagen in yellow, hemoglobin in bright red, cytoplasm and muscle in pink, and nuclei in blue to differentiate structures and identify ELS epithelium.
Primary rabbit polyclonal AQP2 antibodies were purchased from abcam (ab85876; Cambridge, Massachusetts), with dilution of 1:1000 and incubation for 1 hour at RT. Primary rabbit polyclonal V2R antibodies were purchased from Atlas Antibodies (HPA046678; Stockholm, Sweden), with dilution of 1:200 and incubation at RT for 3 hours. Primary rabbit polyclonal NKCC2 antibodies were purchased from Atlas Antibodies (HPA018107), with dilution of 1:100 and incubation at RT for 1 hour. Primary rabbit polyclonal TRPV4 antibodies were obtained from abcam (ab39260), with dilution of 1:50 and incubation at RT for 2 hours.

IHC was carried out using the automated Discovery XT staining platform from Ventana Medical Systems (Roche Group, Tucson, Arizona). Antigen recovery was conducted using heat-induced epitope retrieval, with standard CC2 (Ventana Medical Systems, Oro Valley, Arizona) using a low pH citrate buffer or standard CC1 (Ventana Medical Systems) using a high pH TRIS EDTA buffer.

Primary antibody anti-TRPV4 was detected using the ChromoMap DAB detection kit (Ventana Medical Systems) and OmniMap anti-Rb HRP (Ventana Medical Systems). Primary antibodies anti-AQP2, anti-NKCC2, and anti-V2R were detected using the DABmap detection kit (Ventana Medical Systems) and a Biotin-SP–conjugated Affinipure Donkey Anti-Rabbit IgG (Jackson ImmunoResearch Laboratories, West Grove, Pennsylvania). The anti-Rb horseradish peroxidase (HRP) secondary antibody was applied for 32 minutes at RT. Slides were counterstained and cover-slipped.

Highly Sensitive Pixel Density Analysis

Slides were digitized at ×40 magnification using the NanoZoomer 2.0-HT slide scanner and visualized using the software NDPview2 (Hamamatsu Photonics, Boston, Massachusetts). Epithelial structures were defined as regions of interest (ROIs) on each slide. Each sample size is provided in the form of ROI total surface (Table 1).

HPS-stained slides of the corresponding sample were visualized simultaneously to confirm the epithelium in ROIs of the immunostained slide. Examples are shown in Figure 1 and Figure 2.

Quantitative pixel density analysis was performed with the Visiomorph DP software (Visiopharm, Broomfield, Colorado). The software was internally calibrated to perform cluster analysis and assign pixels to 4 classes: negative (blue), low (yellow), moderate (orange), and high (brown) staining intensities (Figure 1: MD; Figure 2: VS). Mean intensity (MI) and partial and total surface areas were detected by the software, with signal intensity corresponding to pixel density. Global score (GS) for a given ROI was calculated as shown below. Mean GS was generated by combining all ROIs on 1 image, which corresponds to all stained epithelial cells with 1 specific antibody on 1 specific ELS sample. This equation makes it so GS would increase significantly only with a large relative area of concentrated brown pixels (high intensity).

\[
GS = \frac{(MI \times Area)_{\text{Low}} + (MI \times Area)_{\text{Moderate}} + (MI \times Area)_{\text{High}}}{\text{Total ROI Area}}
\]

Statistics

Statistical analysis was completed using SPSS version 24 (SPSS, Inc, an IBM Company, Chicago, Illinois). For continuous normally distributed variables, Student t test was used. For proportions, the χ² test was used. For nonnormally distributed data, the Mann-Whitney nonparametric test was performed. The Shapiro-Wilk test was used to assess normality of each data set. P < .05 was considered significant.

Results

Patient Demographics and Plasma AVP

Mean age, sex distribution, and pAVP for our MD group (n = 24) and VS group (n = 23) are shown in Table 2. Plasma AVP values were well above the upper normal limit of 3.5 pg/mL. Blood samples were obtained from patients the morning of surgery, following 12 hours on nil per os (NPO) orders. There was no statistically significant difference between our groups (P = .55).

Immunohistochemistry: Global Scores for Staining Intensity

Quantitative analysis was performed on immunostained ELS samples. Each primary antibody was evaluated separately, and the software generated a single GS for each sample. In the first year following surgery, 3 operated patients with MD continued to have severe episodic spells of rotatory vertigo, frequently accompanied by migraine-type headaches. Furthermore, they had peculiar auras that preceded some attacks (tingling sensations on the tongue or face), occasionally not followed by vertigo. They were treated with amitriptyline for 3 to 6 months; all 3 were successfully controlled within 4 weeks. We concluded that they had had vestibular migraines and had been misdiagnosed with definite MD, despite satisfying all AAO-HNS criteria. We then identified their AQP2 levels to be the lowest in our MD cohort. In light of these observations, we repeated the analysis with exclusion of the aforementioned 3 patients from the MD group. Global scores are shown in Table 3. For AQP2, the MD group had a mean GS of 90.94, and the VS group had a significantly lower score of 75.83 (P = .003). None of the differences observed for V2R, NKCC2, and TRPV4 were statistically significant.

Total Surface Area of Stained Endolymphatic Sac Epithelium

Mean total areas of analyzed ELS epithelium (ROIs) are shown in Table 3. This represents epithelial structures, confirmed by comparison with HPS-stained slides of the same sample (Figures 1 and 2). Total surface areas of ROIs are summarized in Table 1. AQP2, V2R, NKCC2, and TRPV4 total marked surface areas were not significantly different in MD and VS samples.
Discussion

Plasma AVP, V2R, and Significance in Ménière’s Disease

Experimental insights into the AVP-V2R-AQP2 pathway have raised important questions for understanding MD’s pathophysiology. Several groups mention higher pAVP in MD, particularly during active disease, but many did not incorporate the AAO-HNS diagnostic criteria in their study design. Our results showed that patients with MD and controls respond similarly to dehydration, caused by 12 hours of fasting. We initially expected pAVP elevation in both groups because of mild dehydration and that patients with MD would exhibit an exaggerated response; it was not the case with our cohort (P = .55).

Kumagami et al observed that chronic systemic AVP administration in guinea pigs facilitated EL hydrops. Takeda et al studied the effect of a V2R inhibitor and concluded that V2R activation resulted in EL influx, while inhibition resulted in EL efflux, providing substantial evidence for hyperactivity of this pathway in MD. Kitahara et al reported that V2R and pAVP were elevated in MD, with increased V2R sensitivity to vasopressin, but their experiment did not isolate ELS epithelium. Our results propose a different process: no statistically significant difference was observed in V2R expression, which suggests that its regulation on the ELS may be bypassed altogether: both groups have elevated pAVP, but only MD overexpressed AQP2.

Endolymphatic Sac AQP2: AVP-V2R-AQP2 Axis in Ménière’s Disease

AQP2 is expressed in the human ELS and other sites of perilymph-endolymph (PL-EL) exchange (stria vascularis, Reissner’s membrane) that are potential targets for V2R-AQP2 regulation. The osmotic gradient across the PL-EL barrier must remain tightly controlled in the cochlea for normal hair cell function. The PL reflects capillary osmolarity at 289 mOsm/Kg H2O; EL is maintained at 322 mOsm/Kg H2O. Water flows passively from PL to EL, and this influx must be matched by an efflux somewhere in the inner ear. The experiments by Takeda et al with V2R antagonist have shown that AVP-V2R-AQP2 activation results in EL influx. Given such evidence, there should be a

### Table 1. Mean Total Area of Marked Endolymphatic Sac Epithelium, Adjusted with Exclusion of Vestibular Migraine Cases.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Ménière’s Disease (n = 24)</th>
<th>Controls (VS) (n = 23)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AQP2</td>
<td>66.71 (41.9-91.6)</td>
<td>81.8 (55.3-108.3)</td>
<td>.32</td>
</tr>
<tr>
<td>V2R</td>
<td>59.94 (36.7-83.2)</td>
<td>105.04 (60.2-149.9)</td>
<td>.15</td>
</tr>
<tr>
<td>NKCC2</td>
<td>59.23 (32.3-86.2)</td>
<td>86.32 (57.5-115.1)</td>
<td>.1</td>
</tr>
<tr>
<td>TRPV4</td>
<td>141.78 (97.8-185.8)</td>
<td>154.39 (109.7-119.1)</td>
<td>.64</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; ROIs, regions of interest; VS, vestibular schwannoma.

Figure 1. Ménière’s disease endolymphatic sac. (A) Hematoxylin, phloxine, and Safran (HPS) staining. (B) Aquaporin-2 (AQP2) immunostaining. (C) Visiomorph color-coded staining assigns blue to negative signals, yellow to low-intensity signals, orange to medium-intensity signals, and brown to high-intensity signals. Red or blue dotted lines highlight endolymphatic sac epithelium (arrow). Since it is not obvious by eye to identify the difference in this figure, we selected the clearest uninterrupted section of epithelium to highlight the way the software assigns pixels to best illustrate how the method works.
high suspicion for hyperactivity of this pathway in MD. ELS endolymph has a different osmolarity (229 mOsm) than cochlear EL and is in contact with hypertonic extracellular fluid (290 mOsm): a significant gradient of 60 mOsm could drive water out of the ELS. According to Maekawa et al, AVP induces AQP2 translocation to the basolateral epithelial membrane, altering the luminal membrane’s permeability to water and reducing EL efflux. In our experience, blockage of the EL duct controls MD better than both ELS decompression and intratympanic corticosteroid injections; it would be careless to disregard the possibility that MD pathology originates in the ELS. We speculate that increased AQP2 expression in the ELS epithelium could cause some form of intracellular “overflow” phenomenon, where saturated vesicular AQP2 stores are more sensitive to normal serum AVP fluctuations. Since chronic AVP stimulation is shown to be pathologic in experimental animals, this could contribute to chronic endolymph retention and disease progression. We suspect that blocking the EL duct prevents the diseased ELS from communicating excessive EL volumes to the cochlea.

The present study supports the model by Maekawa et al: overexpression of AQP2 selectively at the ELS basolateral epithelial membrane is expected to produce similar effects to chronic AVP stimulation. A plausible explanation is that AQP2 remains contained within endosomal vesicles until circulating AVP stimulation induces translocation in situations such as dehydration. The resulting changes in membrane AQP2 density would be amplified in MD, and EL hydrops would be facilitated.

This study is a quantitative comparison, not a functional analysis: we cannot make inferences about the activity of the V2R receptor. We propose that if present, a V2R defect must be functional. Loss of inhibition of V2R could cause hydrops by maintaining constant AQP2 expression at the basolateral membrane. Mutations at the active sites of V2R are plausible defects that would provide a satisfactory mechanism for unilateral disease.

It is common practice in MD to attempt systemic diuresis, aiming at reduction of fluid overload, with the assumption that it would also reduce EL hydrops. Accumulating evidence for V2R-AQP2 mechanisms has raised questions about this approach: dehydration leads to increased serum osmolarity and elevation in pAVP. In turn, AVP acts on the inner ear to preserve EL volume and exacerbate hydrops. Takeda et al found that systemic delivery of V2R inhibitors was inferior to round window injection in terms of hydrops reduction, thus confirming that AVP has the physiologic function of protecting EL volume in the face of systemic dehydration. Instead, it has been suggested that inner ear aquaporins would be ideal therapeutic targets for MD. In this study, we report overexpression of AQP2 and propose that the underlying pathology is linked to this water channel. Abnormal AQP2 gene transcription could be a likely explanation, but the mechanism behind it remains unknown. Elevated systemic AVP is unlikely because unilateral disease is the most common form of MD. Increased V2R receptor activity to stimulate de novo AQP2 synthesis

Table 2. Demographics and Plasma AVP.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Menière's Disease (n = 24), Mean (95% CI)</th>
<th>Controls (VS) (n = 23), Mean (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>54.3 (47.5-61)</td>
<td>51.6 (46.1-57.1)</td>
<td>.92</td>
</tr>
<tr>
<td>Sex (% males)</td>
<td>26</td>
<td>47.8</td>
<td>.11</td>
</tr>
<tr>
<td>pAVP, pg/mL</td>
<td>16.66 (0-35.4)</td>
<td>17.84 (5.7-30)</td>
<td>.55</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; pAVP, plasma vasopressin (normal range = 1-3.5 pg/mL); VS, vestibular schwannoma.
is possible: somatic mutations at the receptor site, leading to increased sensitivity to normal AVP levels, or at the inhibitory site, leading to loss of negative feedback inhibition, could both result in increased AQP2.

Finally, vestibular migraine (VM) is a differential diagnosis for MD that is often difficult to rule out on initial presentation. Our experiment shows that VM maybe differentiated histologically, as AQP2 and V2R expression was not significantly different from controls. Clinically, this finding suggests different underlying processes and therefore treatment for these conditions. Furthermore, AQP2 would be an interesting experimental therapeutic target, as localized AQP2 inhibition could theoretically control EL hydrops and disease symptoms. In the future, AQP2 gene therapy could prove useful. For these therapeutic avenues to become a reality, more research is needed to confirm AQP2’s specific involvement in MD’s pathophysiology.

**NKCC2 and TRPV4 in Ménière’s Disease**

In the present study, we did not find altered expression of NKCC2 or TRPV4 in the ELS of patients with MD. On the ELS, luminal membrane NKCC2 drives a Cl⁻-mediated water movement into the cell; a pathology involving NKCC2 could alter the delicate osmotic balance required for ELS functions. Again, membrane expression density is not reflective of functional status. Theoretically, decreased NKCC2 activity could result in lower NaCl reabsorption and weakening of the efflux gradient.

TRPV4 was the last objective of our experiment, and we hypothesized an underexpression on the ELS in MD. TRPV4 is known to drive a Ca²⁺-dependent cellular response to hypotonic stimuli, such as large volumes of hypertonic EL accumulating in the ELS. We could not detect an altered expression of this channel, which suggests EL hydrops is probably not caused by a blunted response to hypotonic cell stress.

**Study Limitations and Future Research**

In our MD group, only a small ELS biopsy was obtained to limit dissection and preserve the posterior semicircular canal integrity. This translates into less total ELS tissue compared to samples obtained from controls. Furthermore, during microtome sectioning, some epithelial structures were sloughed off, which reduced our effective sample size. Finally, the quantitative analysis performed does not evaluate protein function, and the 17% difference observed in AQP2 expression may not be sufficient to explain pathologic fluid shifts. MD is likely multifactorial, and fluid shift analysis is beyond the scope of this article.

Future research should focus on establishing a clear mechanism for AQP2 translocation in human ELS. Other sites of AQP2 expression (stria vascularis, Reissner’s membrane) should also be studied. Finally, genetic research is needed with particular attention to epigenetic processes, promoter regions, splice sites, and other posttranscriptional phenomena involving inner ear aquaporins.

**Conclusion**

In this study, we have demonstrated an increased expression of AQP2 in the ELS epithelium of patients affected by MD, which raises considerable suspicion for involvement of this aquaporin in the pathophysiology. We could not detect an altered protein expression of V2R, NKCC2, or TRPV4 on the ELS. We did not observe pAVP variations that could be attributed to MD.

**Acknowledgments**

We thank the outstanding team at the histology lab of IRIC (Institute for Research in Immunology and Cancer, University of Montreal) for their technical expertise, help, and guidance in conducting our experiments. We also thank Djamal Berbiche for his guidance with the statistical design and review.

**Author Contributions**

Marc-Henri Asmar, protocol, sample collection, lab work, data analysis, manuscript writing, final approval of the V5 (to be published); Louis Gaboury, protocol, pathology lab work, manuscript review and final approval of the V5 (to be published); Issam Saliba, protocol, sample collection, data analysis, manuscript review, final approval of the V5 (to be published).

**Disclosures**

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**References**


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**Table 3. Mean Global Scores for Immunostaining Densities, Adjusted with Exclusion of Vestibular Migraine Cases.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Ménière’s Disease (n = 24)</th>
<th>Controls (VS) (n = 23)</th>
<th>Difference, %</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AQP2</td>
<td>90.94 (77.3-99.3)</td>
<td>75.83 (69-82)</td>
<td>17</td>
<td>.003a</td>
</tr>
<tr>
<td>V2R</td>
<td>22.93 (17.1-27.6)</td>
<td>23.8 (16.6-31)</td>
<td>3.7</td>
<td>.98</td>
</tr>
<tr>
<td>NKCC2</td>
<td>23.87 (18.5-28.1)</td>
<td>23.11 (18.6-27.6)</td>
<td>3.2</td>
<td>.99</td>
</tr>
<tr>
<td>TRPV4</td>
<td>88.4 (75.7-98.4)</td>
<td>88.42 (81.2-98.5)</td>
<td>0.02</td>
<td>.22</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; VS, vestibular schwannoma.

*aStatistically significant.*


