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Authors

Hosokawa, Seiji
Hosokawa, Kumiko
Ishiyama, Gail
et al.

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Immunohistochemical localization of megalin and cubilin in the human inner ear

Seiji Hosokawa[a,b], **Kumiko Hosokawa**[a,b], **Gail Ishiyama**[c], **Akira Ishiyama**[a], and **Ivan A Lopez**[a,*]

[a]Department of Head and Neck Surgery, David Geffen School of Medicine, University of California, Los Angeles,

[b]Department of Otorhinolaryngology/Head & Neck Surgery, Hamamatsu University School of Medicine,

[c]Department of Neurology, David Geffen School of Medicine, University of California, Los Angeles

Abstract

Megalin and cubilin are endocytic receptors expressed in many absorptive polarized epithelia. These receptors have been implicated in the transport of gentamicin in the inner ear as possible contributors to ototoxic damage. Megalin and cubilin have been characterized in detail in the mouse and rat inner ear, but not in the human inner ear. In this study, megalin and cubilin were localized by immunohistochemistry using affinity-purified antibodies in formalin fixed frozen cryostat and celloidin embedded sections of the human inner ear. In the cochlea megalin and cubilin were localized in marginal cells of the stria vascularis, epithelial cells of the spiral prominence and the Reissner's membrane. In the macula utricle and cristae ampullaris, megalin and cubilin were localized in transitional and dark cells, but not in vestibular hair cells and supporting cells. In the endolymphatic duct megalin and cubilin were localized in the epithelial cells. The localization of megalin and cubilin in the human inner ear is consistent with previous reports in the inner ear of animal models and suggest that these receptors may play an important role in the inner ear endocytic transport, and maybe potential targets for prevention of ototoxic damage or the delivery of medications.

Keywords

megalin; cubilin; human inner ear; vestibular end organs; Reissner's membrane; cochlea

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*Corresponding Author: Ivan A. Lopez, Ph.D., ilopez@ucla.edu, 35-64 Rehabilitation Center, 1000 Veteran Avenue, Los Angeles CA 90095, Ph: 310-825-5331.

1. Introduction

Cellular compartmentalization in the inner ear allows for proper hearing and balance function. In the cochlea, the scala vestibuli and the scala tympani contain perilymph and the scala media contains endolymph, with highly different potassium and sodium concentrations and protein composition (Wangemann, 2002; Zdebik et al., 2009). The transport of proteins and other molecules in and out of these compartments is likely to be mediated by megalin and cubilin two multi ligand endocytic receptors found in the apical part of epithelial cells of the kidney and inner ear (Arai et al., 2008; Christensen and Birn, 2002; Eshbach and Weisz, 2017; Gbuker et al., 2002; Ishida et al., 2006; Knipper et al., 2006; Konig et al., 2008; Kozyraki et al., 1998; Mizuta et al., 1999; Moestrup et al., 2001; Tauris et al., 2009; Verroust and Christensen, 2002) and other absorptive epithelia (Nielsen et al., 2016). In the mouse and rat cochlea megalin and cubilin are located in epithelial cells of the Reissner's membrane, stria vascularis, spiral prominence and the endolymphatic sac *and* in transitional and dark cells of vestibular end organs (Arai et al., 2008; Ishida et al., 2006; Konig et al., 2008; Mizuta et al., 1999; Tauris et al., 2009).

Megalin, formerly called gp330, is a 600-kDa transmembrane protein belonging to the low-density lipoprotein (LDL) receptor-related family (Christensen et al., 1992, 2002). Megalin is encoded by *LRP2* (Farquhar et al., 1994; Raychowdhury et al., 1989; Tauris et al., 2009). Megalin was originally identified as the pathogenic autoantigen in Heymann nephritis, a rat model of human membranous nephropathy (Farquhar et al., 1994). Complete cloning and sequencing of megalin identified this molecule as the largest member of the LDL receptor-related protein family (Saito et al., 1994).

Megalin is expressed in several absorptive epithelial cells, including kidney proximal tubules, visceral yolk sac, epididymis, and female reproductive tracts (Christensen and Birn, 2002; Moestrup and Verroust, 2001), and the rat inner ear (Mizuta et al., 1999). Megalin serves as a scavenger receptor and Ca^{2+}-binding receptor (Christensen et al., 1992), and functions to regulate hormone metabolism and vitamin D absorption in cooperation with another receptor, cubilin (Christensen and Birn, 2002). Megalin has also been implicated in the binding of aminoglycosides in the kidney (McWilliam et al., 2017), and inner ear, and maybe involved in ototoxicity. Biallelic pathogenic variants in *LRP2* are associated with the autosomal recessive disorder Donnai-Barrow syndrome and facial-ocular-acoustic renal syndrome (DBS/FOAR) that includes sensorineural hearing loss among other phenotypes (Nielsen et al., 2016).

Cubilin (CUBN) acts as a receptor for intrinsic factor-vitamin B12 complexes, it is also referred as gp280. Cubilin is a 460-kDa peripheral membrane encoded by the *CUBN* (Moestrup et al., 1998). The complete cDNA sequence of cubilin have been characterized in the rat (Moestrup et al., 1998), dog (Xu et al., 1999), and human (Kozyraki et al., 1998). Biallelic pathogenic variants have been associated with megaloblastic anemia that could lead to sensory impairment (Aminoff et al., 1999).

Megalin and cubilin endocytic receptors and their ligands provide epithelial cells with important nutrients (Verroust and Christensen, 2002). Identification of these endocytic

receptors and their potential to transport ligands in the human inner ear may have important clinical application in the development of novel treatment of several inner ear diseases. Given the tightly controlled drug transport of medications through the elaborate blood labyrinthine barrier (Ishiyama et al., 2017; Shi et al., 2016) and epithelial barriers, the identification of megalin and cubilin in the human inner ear may be relevant for the design and administration of drugs that can be delivered via endocytosis in the treatment of human otopathologies.

In the present study megalin and cubilin localization in the human inner ear was investigated by immunohistochemistry using formalin fixed cryostat sections and celloidin embedded sections of the human inner ear. We found that their localization in the human inner ear closely resembled the one found in the inner ear of rodents and suggest the existence of a tightly regulated homeostatic mechanism for endocytic transport mediated by megalin and cubilin.

2. Results

2.1. Megalin and cubilin localization in the human cochlea

Megalin and cubilin were localized by immunofluorescence (-IF) in formalin fixed cryostat sections of the human cochlea microdissected from normal temporal bones obtained at autopsy (no audio-vestibular disorders, Table 1). Megalin and cubilin colocalized in epithelial cells of the Reissner's membrane (Fig. 1). The epithelial cells of the Reissner's membrane form a continuous layer. Megalin-IF (green) was seen in the cytoplasm of epithelial cells (scala media) (Fig. 1a). Cubilin-IF (red) was also seen in these epithelial cells (Fig. 1b). Merged image, shows that both megalin and cubilin colocalized in Reissner's membrane epithelial cells (yellow color), few cells were cubilin-IF only (red color) (Fig. 1c). Higher magnification view shows the punctated megalin and cubilin immunofluorescence signal (Fig. 1d), yellow color indicated colocalization. DAPI (in blue) allowed the identification of cell nuclei. Mesenchymal cells located at the scala vestibuli were non-IF for megalin and cubilin.

Megalin-IF was also seen in epithelial cells of the spiral prominence and marginal cells of the stria vascularis (Fig. 2a, green color) and cubilin-IF (Fig. 2b, red color). Merged image (from Fig. 2a and Fig. 2b) shows colocalization of megalin and cubilin (Fig. 2c, yellow color). Higher magnification view of the spiral prominence (from Fig. 2c), shows in detail the localization of both proteins, cells in the spiral ligament were non immunoreactive (Fig. 2d). Inner and outer hair cells and supporting cells in the organ of Corti and spiral ganglia neurons were non-IF to both megalin and cubilin.

Megalin and cubilin immunolocalization was also investigated in formalin fixed celloidin embedded sections of the human cochlea using secondary antibodies labeled with horseradish peroxidase and visualized with diaminobenzidine (HRP-DAB) (Fig. 3). Megalin-IR and cubilin-IR were found in the Reissner's membrane, marginal cells of the stria vascularis and spiral prominence (Fig. 3a and Fig. 3b respectively). Intermediate and basal cells of the stria vascularis and cells in the spiral ligament were nonimmunoreactive

for both megalin and cubilin. All human cochleas immunostained using fluorescent or HRP labeled secondary antibodies exhibited a similar megalin and cubilin distribution (Table 1).

2.2   Megalin and cubilin immunofluorescence (IF) in the human vestibular end organs

Megalin-IF and cubilin-IF was found in formalin fixed cryostat sections of the human cristae ampullaris and macula utricle microdissected from temporal bones (Table 1). Megalin-IF (green color) was seen in the apical portion of transition and dark epithelial cells located outside the crista sensory epithelia (Fig 4a). Cubilin-IF (red color) was seen in the cytoplasm of transitional and dark cells (Fig. 4a1). The crista hair cells and supporting cells were non-IF for both megalin and cubilin; this also was true for the stromal cells, nerve fibers and terminals and vascular tissue located underneath the crista sensory epithelia. Colocalization of megalin and cubilin is seen in Fig. 4a2 (merged image from Fig. 4a and 4a1). Higher magnification view shows the specific localization (yellow color) of both megalin and cubilin in the apical portion of transitional and dark epithelial cells (Fig. 4a3). A similar pattern of megalin and cubilin immunofluorescence was seen in the macula utricle. Megalin (green color) and cubilin (red color) were found in the transitional and dark cells (Fig. 4b and 4b1, respectively). Merged images show colocalization of both proteins (Fig. 4b2). Higher magnification view of transitional cells showed specific immunofluorescence for both megalin and cubilin at the apical portion of transitional epithelial cells (Fig. 4b3). Like in the cristae vestibular hair cells, supporting cells and stromal cells underneath the utricle sensory epithelia were non-IF for both megalin and cubilin.

Megalin and cubilin immunoreactivity (-IR) was also investigated in celloidin embedded sections of the crista ampullaris and macula utricle using HRP-DAB (Fig. 5). Both megalin-IR and cubilin-IR were localized in the transitional and dark cells of the crista (Fig. 5a and Fig. 5a1) and macula utricle (Fig. 5b and Fig. 5b1). The cristae and macula utricle hair cells and supporting cells and the stroma were non-IR for megalin and cubilin. All human crista and utricle immunostained using fluorescent or HRP labeled secondary antibodies exhibited a similar megalin and cubilin distribution (Table 1).

2.3.   Megalin and cubilin immunoreactivity in the human endolymphatic sac

Megalin-IR and cubilin-IR in the endolymphatic sac was investigated in celloidin embedded sections from normal temporal bones (Table 1). Megalin-IR and cubilin-IR was found in epithelial cells of the endolymphatic duct (Fig. 6a and 6b respectively).

2.4.   Megalin and cubilin immunohistochemical controls

Positive immunohistochemical controls: Formalin fixed cryostat sections of the mouse vestibule and cochlea, and human kidney sections were incubated with antibodies against megalin and cubilin. Megalin-IF was seen in transitional cells of the mouse crista ampullaris (Fig. 7a), and cubilin-IF was detected in epithelial cells of the mouse Reissner's membrane (Fig. 7b). Megalin-IR was found in epithelial cells of the proximal tubule of the human kidney (Fig. 7c). The pattern of immunoreactivity found in the positive controls for both receptors was similar to that reported previously (Tauris et al., 2009).

Negative immunohistochemistry controls: Human celloidin sections were incubated in the absence of primary antibodies or using the primary antibodies (megalin or cubilin) pre-adsorbed with their corresponding antigen. No specific megalin or cubilin immunoreactivity was seen in the negative controls (Fig 7d, 7d1 and 7d2 respectively).

3. Discussion

Megalin and cubilin were localized by immunohistochemistry using formalin fixed frozen sections of the cochlea and vestibular end organs microdissected from temporal bones and celloidin-embedded sections of the human inner ear. Specifically, megalin and cubilin immunoreactivity was seen in marginal cells of the stria vascularis and epithelial cells of the Reissner's membrane. In the crista and macula utricle, megalin and cubilin immunoreactivity was seen at the apical surface and subapical area of transitional and dark cells.

Megalin and cubilin distribution in the human inner ear resembles the one previously reported in the mouse and rat inner ear (Arai et al., 2008; Ishida et al., 2006; Konig et al., 2008; Tauris et al., 2009). This suggest that both proteins may function as endocytosis receptors involved in endolymphatic homeostasis (Arai et al., 2008; Mizuta et al., 1999; Tauris et al., 2009). Immuno-electron microscopic studies are necessary to better understand the functional role of megalin and cubilin in the human inner ear.

Megalin and cubilin endocytosis may play a role in ototoxicity mediated by cisplatin or gentamicin. Megalin is highly expressed in the proximal tubular cells of the kidney and marginal cells of the stria vascularis suggesting that this receptor may play a role in the high sensitivity of these organs to cisplatin-induced toxicity. Hammond et al., (1997) suggested that uptake of aminoglycosides in proximal tubule cells involves the megalin-mediated endocytic pathway. In this respect blockage of megalin has been associated with suppression of nephrotoxicity mediated by gentamicin, vancomycin or cisplatin (Hori et al., 2017), and it has been suggested that single nucleotide polymorphisms (SNPs) in *LRP2* may impact the individual susceptibility against cisplatin-induced ototoxicity (Riedemann et al., 2008). It is important to mention that there are other mechanisms of ototoxicity via the uptake and metabolism of aminoglycosides by auditory and vestibular hair cells (Lim., 1986; Richardson et al., 1997). Another role for megalin and cubilin located in the epithelial cells of the Reissner's membrane may be participating in the endocytosis of debris accumulated in the endolymph after acoustic trauma (Hunter-Duvar, 1978).

Megalin and cubilin immunoreactivity was seen in epithelial cells of the human Reissner's membrane and the spiral prominence membrane as well as strial marginal cells. The Reissner's membrane is positioned between endolymphatic fluid and perilymphatic fluid (Ikeda and Morizono, 1991), thus, the localization of megalin and cubilin receptors in the Reissner's membrane support their role in maintaining homeostasis of the endolymph (Mizuta et al., 1999; Arai et al., 2008; Tauris et al., 2009).

Megalin and cubilin were also detected in epithelial cells of the spiral prominence. It has been suggested that the spiral prominence may play a role in the fluid homeostasis of the

cochlear duct, acting in concert with the stria vascularis and Reissner's membrane (Santi et al., 2015). Pendrin is an anion transporter that exchanges chloride for bicarbonate located also in the luminal face of the epithelial cells of the spiral prominence (Yoshino et al., 2006). This suggest that megalin and cubilin as well was pendrin may play an important role in maintaining the endolymphatic anion balance.

Megalin and cubilin were also found in the marginal cells of the human stria vascularis. A study by Knipper et al., (2006), showed that a LIMP2-deificient mice display progressive high-frequency hearing loss and decreased otoacoustic emission as early as 4 weeks of age, due to loss of the potassium channel KCNQ1 and the beta-subunit of the KCNE1 channel in the marginal cells parallel with a decrease in the expression of megalin in the luminal surfaces of marginal cells of the stria vascularis.

Megalin and cubilin immunoreactivity was detected at the apical surface, and subapical area of the vestibular dark cells, suggesting that they may participate in the transport of nutrients and ligands in the vestibular epithelium. Vestibular dark cells and strial marginal cells play important roles in maintenance of endolymph within the vestibular system (Arai et al., 2008; Ishida et al., 2006). Several ion channels, co-transporters, and ATPases, involved in K^+ recycling, have been previously been detected in vestibular dark cells and strial marginal cells (Wangemann, 2002), suggesting that they may work in concert with the megalin and cubilin receptors to regulate fluid and ion balance.

Megalin and cubilin were also found in epithelial cells of the human endolymphatic sac. Ishida et al., (2006), found megalin in the lumen of endolymphatic sac. Megalin detected in the rat endolymphatic sac was of the smaller molecular weight type, lacking a cytoplasmic domain. Rask-Andersen et al. (1991), detected glycoprotein secretion in human endolymphatic sac epithelial cells, suggesting that soluble megalin may be secreted by endolymphatic sac epithelial cells. Ishida et al., (2006) reported megalin-IR in the tectorial membrane suggesting that the tectorial membrane secretes soluble megalin. In contrast to this findings we did not detected megalin in this structure.

We have previously described in detail the advantages and disadvantages of using human inner ear tissue for immunohistochemistry (Lopez et al., 2016). Among the disadvantages is the limited availability of normal human inner ear tissue specimens (temporal bone donors). For this reason, we did not perform quantitative immunohistochemistry to investigate changes with age, gender, or disease condition. It is always important take into consideration several factors that may affect the immunoreactive signal using human tissue, for example agonal state, postmortem time, type of fixative and embedding media (i.e. celloidin embedding). In this study we used normal tissue with an average of 12 hours postmortem.

To validate our results, we did immunohistochemistry and immunofluorescence staining in both formalin frozen sections and celloidin embedded sections with the expectation to obtain similar immunoreactive patters using the two types of tissue processing. The results were consistent with both types of inner ear tissue used and both immunostaining protocols (immunofluorescence and immunohistochemistry). In addition, we compared megalin and cubilin immunolocalization in the human inner ear with reports of megalin and cubilin

distribution in the rat and mouse inner ear. The distribution of both megalin and cubilin was mainly similar, i.e. localized in the apical portion of the epithelial cells, in addition we detected megalin in the cytoplasm of the transitional and epithelial cells. Immunoelectron microscopy in well preserved human inner ear (i.e. surgical tissue) would be helpful to determine the subcellular distribution of both receptors.

In conclusion, megalin and cubilin were found in epithelial cells of the human cochlea and vestibular end organs. Their localization resembles the one detected previously in the inner ear of rodents suggesting and important role in the transport of nutrients and ototoxic drugs. Further ultrastructural studies are indicated to address the functional role of megalin and cubilin immunoreactivity in the human inner ear and also their potential role in ototoxicity.

4. Material and methods

4.1. Specimens

Approval to acquire human tissue was obtained from the University of California, Los Angeles Institutional Review Board (IRB# 10–001449). The temporal bone donors in the present study were part of a National Institute of Health funded Human Temporal Bone Consortium for Research Resource Enhancement (Table 1). All specimens used are from subjects with a documented history of normal auditory and vestibular function. Auditory and vestibular end organs microdissected from temporal bones obtained at autopsy (n=4 ages 70–91 years-old, male and female), and formalin-fixed archival celloidin-embedded human temporal bone sections (n=6, ages 54–84 years-old, male and female) were used (Table 1).

4.2. Temporal bone removal and inner ear tissue processing:

The methods for temporal bone harvesting, tissue fixation and microdissection of vestibular and auditory end organs have been described in detail (Lopez et al., 2005, Lopez et al., 2016). Microdissected crista ampullaris, macula utricle and cochlea were immersed in 30% sucrose in phosphate buffered saline solution (PBS) for 7 days. Before sectioning, the end organs were embedded in Tissue-Tek® and placed on Teflon embedding molds (Polysciences) and properly oriented under the dissecting microscope. Twenty-micron thick cryostat sections were obtained using a Leica cryostat (CM1850) and mounted in Super frost plus slides (Fisher Scientific).

4.3. Megalin and cubilin immunofluorescence (IF) in formalin fixed frozen sections

Tissue sections were incubated at room temperature for 90 minutes 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. At the end of the incubation, the blocking solution was removed and a mixture of the primary polyclonal antibodies was applied. Megalin (H-245) (1:500, Cat. # sc-25470, affinity purified rabbit polyclonal antibody, raised against amino acids 4411– 4655 of megalin of human origin, Santa Cruz Biotechnology, Santa Cruz, CA), and cubilin (Y-20) (1:500, Cat. # sc-20607, affinity purified goat polyclonal antibody raised against a peptide mapping near the C-terminus of cubilin of human origin, Santa Cruz Biotechnology, Santa Cruz, CA) were applied for 48 hours at 4°C.

Following a 3 ×15 minutes PBS washing step, sections were incubated in a mixture of secondary antibodies (1: 1000): donkey anti-rabbit antibody labelled with Alexa 488, and donkey anti-goat antibody labeled with Alexa 594 (Life Sciences) for 2 hours. Then the tissue sections were washed with PBS (3 × 15 minutes) and covered with aqua soluble mounting media (Prolong diamond antifade mountant, Invitrogen) containing DAPI to visualize cell nuclei.

4.4   Megalin and cubilin immunohistochemistry in formalin fixed archival celloidin sections

Immunohistochemistry was performed on formalin fixed celloidin embedded sections. The methodology for celloidin removal and antigen retrieval steps has been described in detail (Lopez et al., 2016). Tissue sections were incubated for one hour with a blocking solution containing 5% normal goat serum/1% bovine serum albumin (BSA) fraction-V (Sigma, St. Louis, MO) and 0.5% Triton X-100 (Sigma) in PBS. Incubation with primary antibodies against megalin and cubilin was performed for 48 hours at 4°C in a humid chamber. The sections were washed with PBS (3 ×15 minutes), and then incubated for one hour with either the goat anti-rabbit or the donkey anti-goat biotinylated secondary antibodies (1:1000, Vector Labs, Burlingame, CA), and then washed with PBS (3 × 15 minutes). Next, one-hour incubation was performed with Vectastain Elite ABC reagent (Vector Labs) followed by PBS washes (15 minutes × 3). Immunoperoxidase staining was performed using Immpact DAB solution (Vector Labs). The reaction was stopped with distilled water washes (15 minutes × 4). Slides were mounted with Vectamount AQ aqueous mounting media (Vector Labs) and glass coversliped.

4.4.1.   Immunohistochemical controls.—Cryostat sections from human kidney and the mouse inner ear were incubated with the antibodies against megalin and cubilin (positive controls). These sections were subjected to the same protocol. As negative control, the primary antibodies against megalin or cubilin were omitted and the immunoreaction was performed in the human cochlea sections as described above, no immunoreaction was observed. Antibody absorption negative controls consist of applying the antibodies against megalin or cubilin pre-absorbed with their corresponding antigen (megalin blocking peptide cat # sc-25470P, and cubilin blocking peptide cat # sc-20607P, both from Santa Cruz Biotechnology, Santa Cruz, CA) in the human cochlea sections (1μg/1μl).

4.5   Microscopic observation and documentation

Immunoreacted tissue sections were examined with an Olympus BX51 fluorescent microscope (Olympus America Inc, NY, USA) equipped with an Olympus DP70 digital camera. Digital images were acquired using MicroSuite™ Five software (Olympus America Inc). All images were prepared using the Adobe Photoshop software program run in a Dell Precision 380 computer.

Acknowledgments

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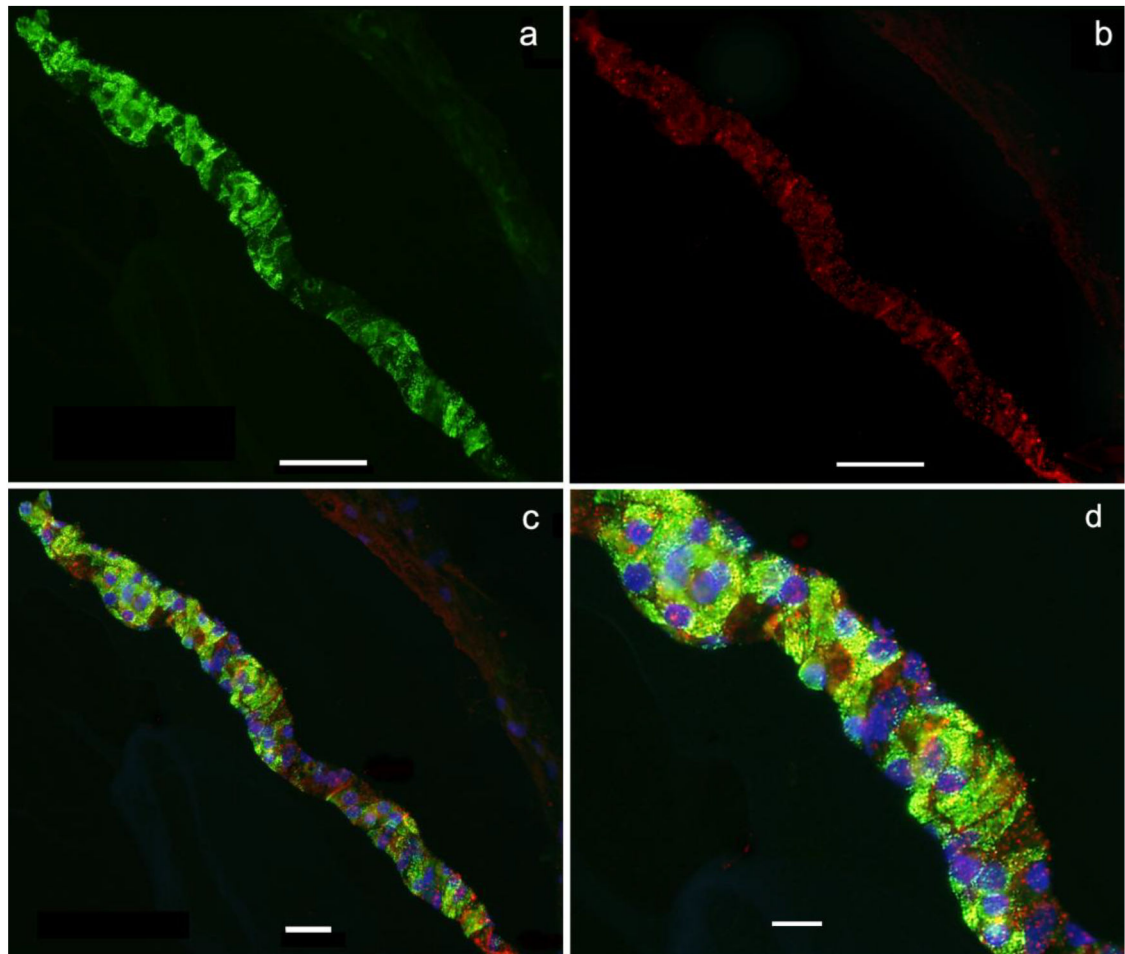
Fig 1.
Megalin and cubilin immunofluorescence (-IF) in a formalin fixed frozen cryostat section of the Reissner's membrane. (a) Megalin-IF in epithelial cells of the Reissner's membrane (green color), (b) cubilin-IF was also detected in the epithelial cells of the Reissner's membrane (red color). (c) This micrograph is a merge image from (a) and (b) showing the colocalization of megalin and cubilin (yellow color). (d) Higher magnification view from (c). DAPI stains cell nuclei (blue color). Scale bar in a and b = 40 μm, c= 20 μm, d= 20 μm.
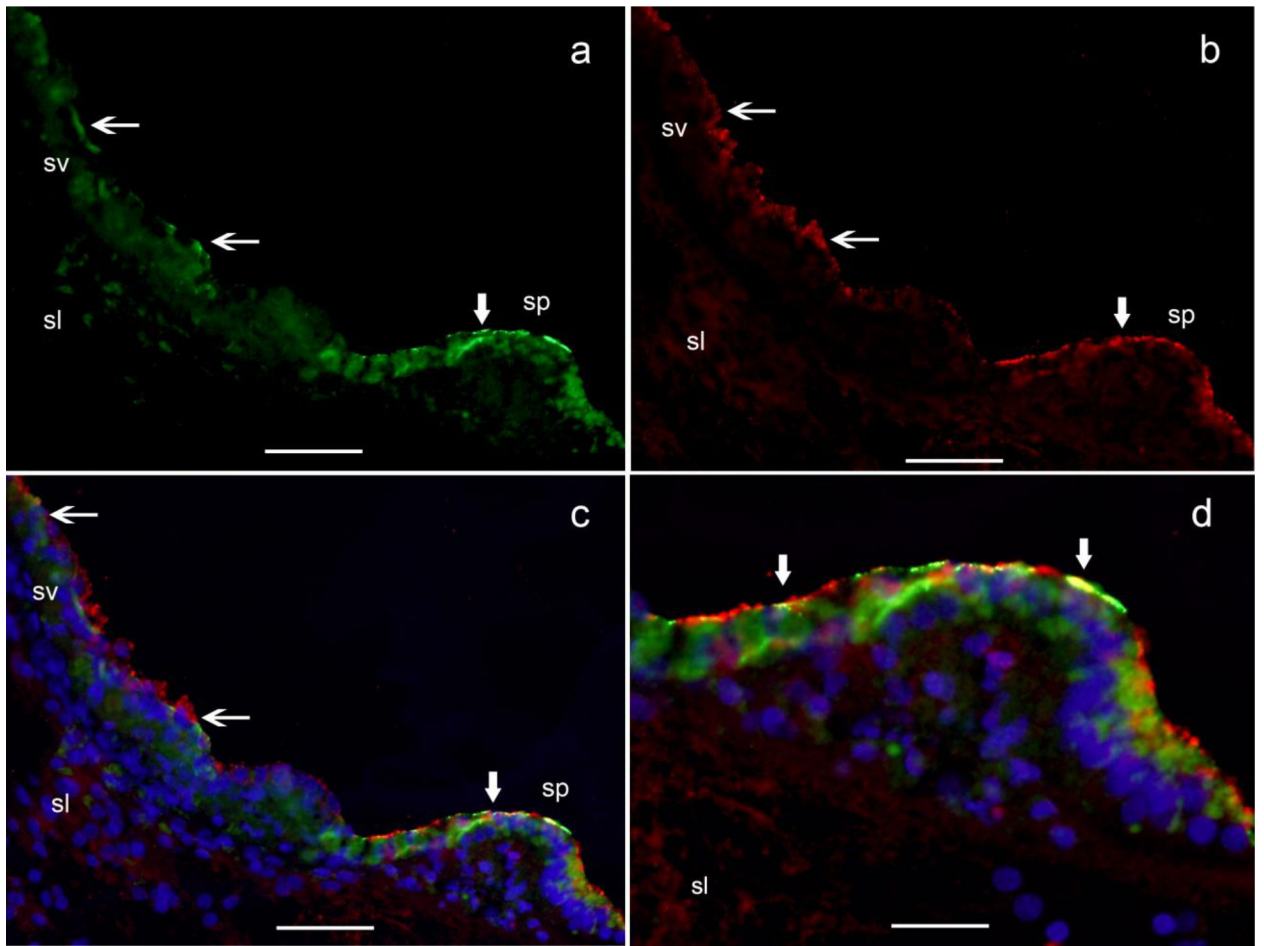
Fig 2.
Megalin-IF and cubilin–IF in the stria vascularis (sv) and spiral prominence (sp). (a)
Megalin-IF (green), thin arrows point to the marginal cells in the sv, thick arrowhead point
to epithelial cells of the sp, the spiral ligament (sl) was no immunoreactive for both proteins.
(b) Cubilin-IF (red), shows a near identical distribution. (c) Merged image from (a) and (b).
(d) High magnification view of the sp shows the specific localization of megalin and cubilin.
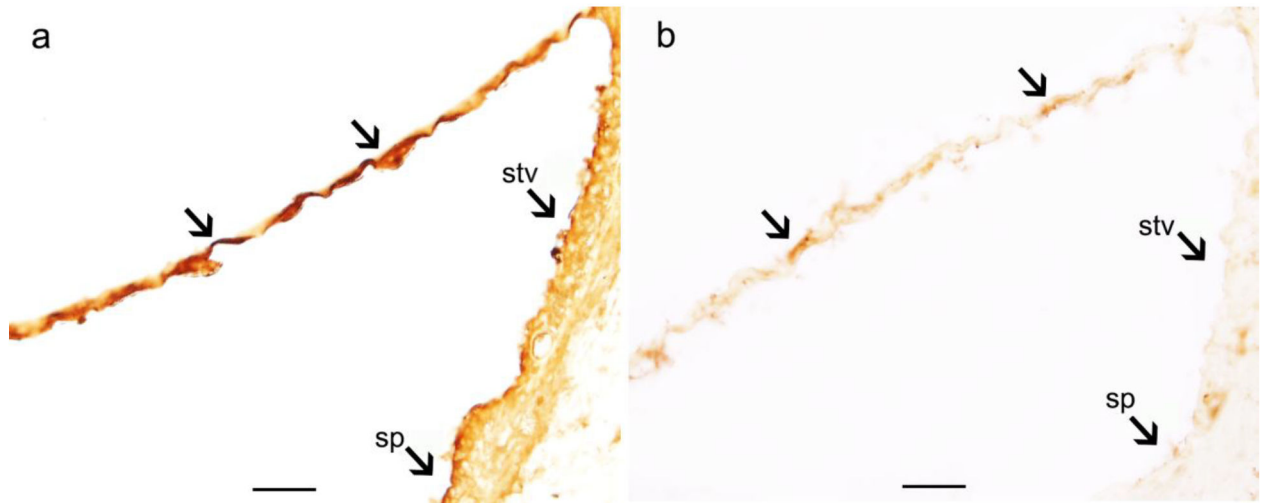Cell nuclei in blue (DAPI). Sl: Scale bar in a, b and c = 60 μm, d= 25 μm.

Fig 3.
Megalin and cubilin immunoreactivity (-IR) in the human cochlea (celloidin embedded temporal bone section). (a) Megalin-IR was seen in the Reissner's membrane (arrows), the stria vascularis and the spiral prominence (arrows). (b) Cubilin-IR showed a similar distribution to megalin. Scale bar = 70 μm.
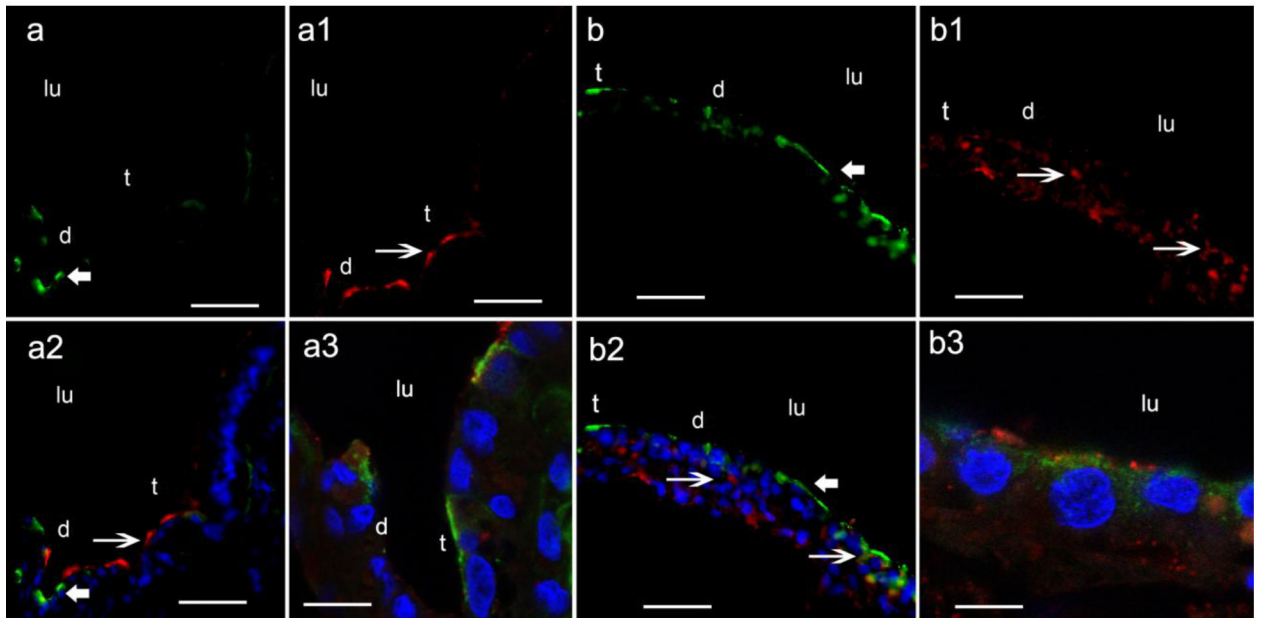
Fig 4.
Megalin-IF and cubilin-IF in formalin fixed cryostat section of the human crista ampullaris and macula utricle. (a) Megalin-IF (green color, thick arrow) is present in transitional (t) and dark cells (d). (a1) Cubilin-IF (red color, long arrow) followed a similar pattern to megalin-IF. (a2) Image merged from (a) and (b) to show colocalization of megalin and cubilin. (a3) High magnification view of the transitional and dark cells from another specimen, showing colocalization of both megalin and cubilin. (b) Megalin-IF and (b1) cubilin-IF in transitional and dark cells of the macula utricle, (b2) Merged image from (b) and (b1). (b3) High magnification view of the transitional cells from another specimen, showing colocalization of both megalin and cubilin, lu: lumen. Scale bar in a,a1 and a2 and b, b1 and b2 = 50 μm, bar in a3 and b3 =10 μm.
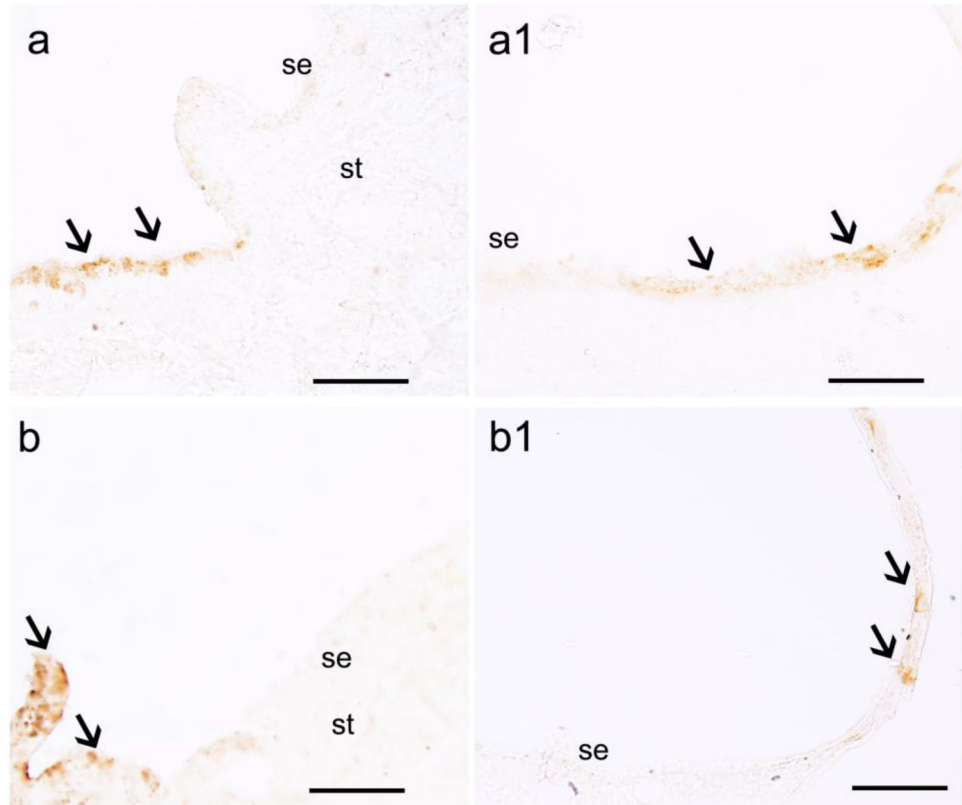
Fig 5.
Megalin and cubilin -IR in celloidin sections of vestibular end organs. (a) megalin-IR was
seen in transitional cells and dark cells (arrows) in the crista ampullaris, (a1) and macula
utricle. The sensory epithelia (se) and stroma (st) was not immunoreactive. (b) cubilin-IR in
the crista ampullaris and (b1) macula utricle (arrows) were found also in the transitional and
dark cells of the macula utricle. Scale bar = 40 μm.

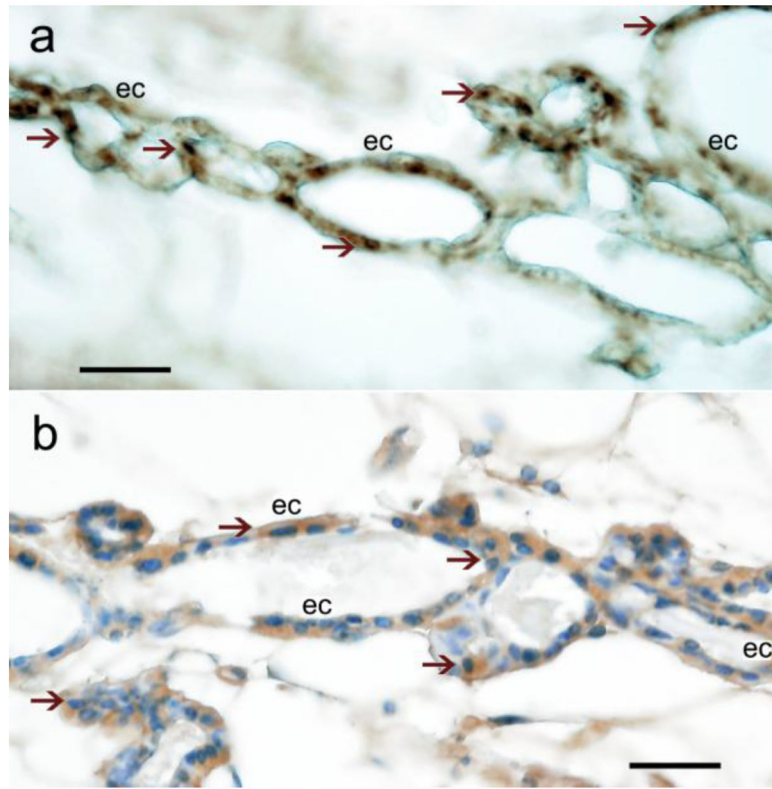**Fig 6.**
Megalin-IR and cubilin-IR in the endolymphatic sac (formalin fixed celloidin embedded sections). (a) Megalin-IR and (b) cubilin-IR was seen in epithelial cells (ec) of the endolymphatic duct (dark amber color, arrowheads). Hematoxylin counterstained (blue color in cell nuclei). Scale bar = 40 μm.
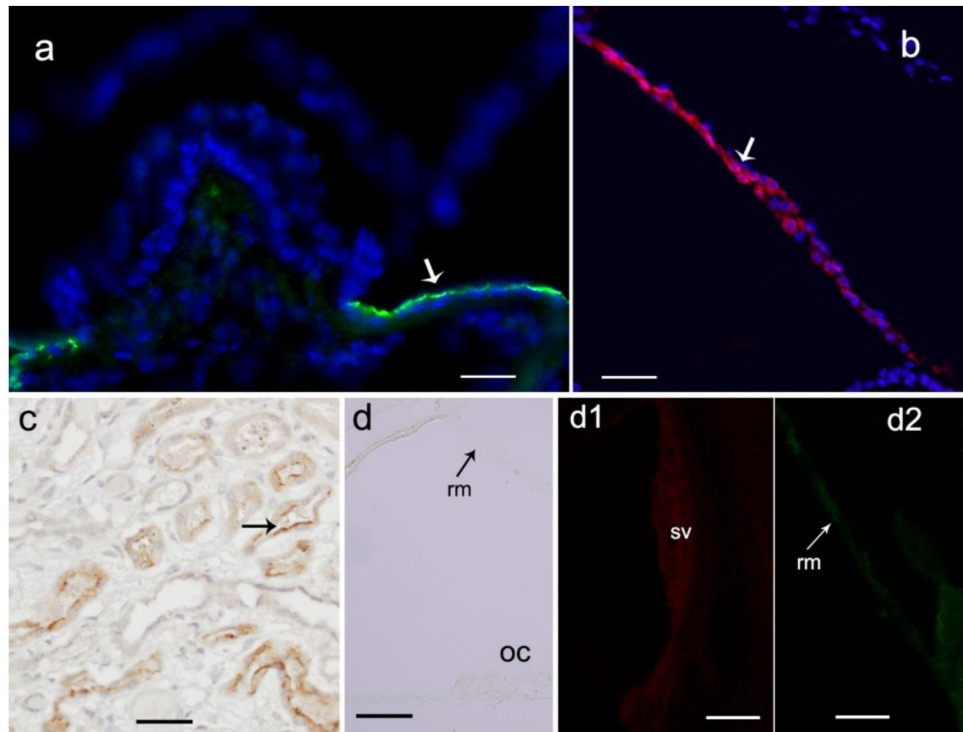
Fig 7.
Megalin-IR and cubilin-IR in positive and negative controls. (a) Megalin-IF (green) in vestibular dark cells flanking the mouse crista ampullaris (arrow). (b) Cubilin-IF (red) in epithelial cells of the mouse Reissner's membrane (arrow), (c) Megalin-IR in epithelial cells (arrow) of a human kidney section (positive control). (d) Negative control in a celloidin embedded section of the human cochlea, primary antibody was omitted, no immunoreactivity was detected. Celloidin embedded sections were incubated with (d1) Antibodies against megalin (sv: stria vascularis) and (d2) cubilin (rm: Reissner's membrane) pre-absorbed with their corresponding antigen. No specific immunoreactivity was seen in both cases. oc: organ of Corti. Scale bar in a and b = 15 μm, c = 60 μm, d = 40 μm, d1 and d2= 50 μm.

Table 1.

Temporal bones used in this study.

Specimen #	Age (years)/gender	PMT	Type of section	Staining type	Figure
1/R	70/F	13	FFF	IF	1a-d, 4b3
2/L	80/M	12	FFF	IF	2a-d.4b.4b1.4b2
3/L	82/M	14	FFF	IF	4a, 4a1, 4a2
4/L	91/F	10	FFF	IF	4a3, 7d1,7d2
5/L	54/F	12	celloidin	HRP-DAB	3a
6/R	79/M	14	celloidin	HRP-DAB	3b
7/R	67/F	12	celloidin	HRP-DAB	5a, 5a1
8/R	71/M	9	celloidin	HRP-DAB	5b, 5b1
9/R	76/F	12	celloidin	HRP-DAB	6a, 7d
10/L	84/F	9	celloidin	HRP-DAB	6b

Abbreviations: R: right side, L: Left side; M: male, F: Female, PMT: post mortem time in hours. All inner ear sections used in this study were from temporal bone donors with normal hearing and balance. FFF: Formalin (10% buffered) fixed frozen cryostat sections. HRP-DAB: immunohistochemistry using horseradish peroxidase and diamino-benzidine, IF: immunofluorescence. Figure: indicates specimen used for these micrographs.

Megalin and cubilin immunoreactivity (IR) was found in the human cochlea and vestibule.

Megalin-IR and cubilin-IR was seen in epithelial cells of the Reissner's membrane and spiral prominence.

Megalin-IR and cubilin-IR was seen in the transitional and dark cells of vestibular end organs.

Both megalin and cubilin may play an important role in inner ear endocytic transport.