UPPSALA UNIVERSITET

Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine 1647

The Human Vestibular Aqueduct, Endolymphatic Duct and Sac

A Morphological Study Using Micro-CT, Super Resolution Immunohistochemistry and Synchrotron Phase Contrast Imaging

CHARLOTTA KÄMPFE NORDSTRÖM

ACTA
UNIVERSITATIS
UPSALIENSIS
UPPSALA
2020

ISSN 1651-6206
ISBN 978-91-513-0901-9
urn:nbn:se:uu:diva-406848

Dissertation presented at Uppsala University to be publicly examined in Enghoffsalen, Akademiska sjukhuset, Ing 50, Uppsala, Thursday, 7 May 2020 at 09:00 for the degree of Doctor of Philosophy (Faculty of Medicine). The examination will be conducted in Swedish. Faculty examiner: Professor Claes Möller (Örebro Universitet).

Abstract
Kämpfe Nordström, C. 2020. The Human Vestibular Aqueduct, Endolymphatic Duct and Sac. A Morphological Study Using Micro-CT, Super Resolution Immunohistochemistry and Synchrotron Phase Contrast Imaging. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine* 1647. 71 pp. Uppsala: Acta Universitatis Upsaliensis. ISBN 978-91-513-0901-9.

The inner ear lies sheltered in the temporal bone and consists of basically three parts: the cochlea (the hearing organ), the vestibular (the balance organ), and the endolymphatic duct (ED) and endolymphatic sac (ES). The ES and ED are located in a bony canal, the vestibular aqueduct (VA), located on the medial side of the vestibule. While the functions of the cochlea and the vestibular part of the inner ear are rather well studied, our knowledge of the function/s of the ES and ED remains limited and has intrigued scientists for centuries. Earlier studies have supported several theories, such as being an immune mediator, an aid in pressure regulation, related to the absorption of endolymph, and the production of endolymph.

Otologic disorders, which affect both hearing and balance, such as Meniere's disease (MD) and large vestibular aqueduct syndrome (LVAS), have been linked to dysfunction of the ES/ED. Studies of the human inner ear are fairly sparse. Research on the ES and ED have mainly been performed on animals, although both the anatomy and function may differ among various species.

This thesis aims to further investigate the anatomy and function of the human ES and ED with the two otologic disorders MD and LVAS in mind. To achieve this, we have used novel imaging techniques, such as super-resolution structured illumination microscopy (SR-SIM), micro-computerized tomography (micro-CT), and synchrotron radiation phase-contrast imaging (SR-PCI). The material used for imaging comes from different sources: human archival temporal bones from the Uppsala temporal bone collection; human fresh-frozen cadaveric bones from our collaborators at Western University, in London, Ontario, Canada; and fresh-frozen human ES harvested during vestibular schwannoma surgery after securing ethical permission.

The results of these studies describe the micro-anatomy of the VA, ED and ES down to a nanoscopic level. The discussion is based on the findings, relating them to earlier research with clinical implications regarding MD and LVAS.

Keywords: Endolymphatic sac, endolymphatic duct, vestibular aqueduct, Meniere's disease, LVAS, micro-CT, synchrotron phase contrast imaging

Charlotta Kämpfe Nordström, Department of Surgical Sciences, Otolaryngology and Head and Neck Surgery, Akademiska sjukhuset, Uppsala University, SE-75185 Uppsala, Sweden.

ISSN 1651-6206
ISBN 978-91-513-0901-9
urn:nbn:se:uu:diva-406848 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-406848)

To my family

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I Nordstrom, C.K., Laurell, G., Rask-Andersen, H. The Human Vestibular Aqueduct: Anatomical Characteristics and Enlargement Criteria. *Otol Neurotol.* 2016 Dec;37(10):1637-1645

II Kämpfe Nordström, C., Danckwardt-Lilliestrom, N. Laurell, G., Liu, W., Rask-Andersen H. The Human Endolymphatic Sac and Inner Ear Immunity: Macrophage Interaction and Molecular Expression. *Front immunol.* 2019 Feb 1;9:3181

III Nordstrom, C.K., Danckwardt-Lilliestrom, N., Liu, W., Rask-Andersen, H. "Reversed polarization" of Na/K-ATPase-a sign of inverted transport in the human endolymphatic sac: a super-resolution structured illumination microscopy (SR-SIM) study. *Cell Tiss Res.* 2020 Mar;379(3):445-457. Epub 2019 Nov 12

IV Kämpfe Nordström, C., Li, H., Ladak, HM., Agrawal, S. & Rask-Andersen, H. A Micro-CT and Synchrotron Imaging Study of the Human Endolymphatic Duct with Special Reference to Endolymph Outflow and Meniere's Disease. Under revision in Nature Scientific Reports.

Contents

Abbreviations

AC	Accessory canal
CC	Common crus
CD	Cochlear duct
CT	Computed tomography
EA	External aperture
ED	Endolymphatic duct
EH	Endolymphatic hydrops
EP	Endocochlear potential
ES	Endolymphatic sac
LVAS	Large vestibular aqueduct syndrome
MD	Meniere's disease
MRI	Magnetic resonance imaging
MR	Mineralocorticoid receptor
SNHL	Sensorineural hearing loss
SR-PCI	Synchrotron radiation phase contrast imaging
SR-SIM	Super-resolution structured illumination microscopy
VA	Vestibular aqueduct
VVA	Vein of the vestibular aqueduct

Introduction

The inner ear harbors the end-organs of two of our sources of sensory input, namely hearing and balance. Dysfunction of one or both of these sensory organs is common and can have a great impact on the ability to perform activities of daily living. When hearing fails, communication is affected. Communication, although consisting of more elements than hearing, is categorically crucial to humans, and hearing is essential for developing a verbal language. Investigations performed by Rosenhall et al. [1], showed that 10.7% of the Swedish population suffers from some kind of hearing loss, and about 30–50 children are born deaf each year in Sweden. Dysfunction of the balance, or vestibular, organ affects our ability to maintain body position and to move around freely while keeping our gaze steady. Dizziness, including a wide range of peripheral vestibular disorders, has been reported to affect up to 20% of the adult population annually [2] and has a considerable impact on health-related quality of life [3]. Inner ear disturbances are the main causes of permanent hearing loss and account for approximately one-quarter of dizziness complaints [2].

The inner ear lies sheltered in the temporal bone and consists of basically three parts: the cochlea (the hearing organ), the vestibular (the balance organ), and the endolymphatic duct (ED) and endolymphatic sac (ES). While the functions of the cochlea and the vestibular part of the inner ear are rather well studied, our knowledge of the function/s of the ES and ED remains limited and has intrigued scientists for centuries. Earlier studies have supported several theories, such as being an immune mediator [4-6], an aid in pressure regulation [7,8], related to the absorption of endolymph [9,10], and the production of endolymph [11-14].

Otologic disorders, which affect both hearing and balance, such as Meniere's disease (MD) and large vestibular aqueduct syndrome (LVAS), have been linked to dysfunction of the ES/ED. The sheltered position of the inner ear is, indeed, beneficial, as it is protected by dense bone, but this also makes the inner ear difficult to study, so studies of the human inner ear are fairly sparse. It is usually difficult to have access to either fresh cadaveric or surgically removed inner ears, and in vivo studies on human material are practically impossible to perform. Therefore, research on the ES and ED have mainly been performed on animals, although both the anatomy and function may differ among various species.

This thesis aims to further investigate the anatomy and function of the human ES and ED with the two otologic disorders MD and LVAS in mind. To achieve this, we have used novel imaging techniques, such as super-resolution structured illumination microscopy (SR-SIM), micro-computerized tomography (micro-CT), and synchrotron radiation phase-contrast imaging (SR-PCI). The material used for imaging comes from different sources: human archival temporal bones from the Uppsala temporal bone collection; human fresh-frozen cadaveric bones from our collaborators at Western University, in London, Ontario, Canada; and fresh-frozen human ES harvested during vestibular schwannoma surgery after securing ethical permission.

Background

Anatomy and histology of the Endolymphatic Duct and Sac

The sensory parts of the inner ear, the cochlea, the vestibular apparatus, and the ES, are all connected as parts of the so-called membranous labyrinth. Inside this compartment, there is a fluid called endolymph, which bathes the surface of the organ of Corti and the apical poles of the hair cells. Another type of fluid, the perilymph, with an ionic composition similar to that of plasma and cerebrospinal fluid, is located between the bony capsule and the membranous labyrinth.

The ES and ED are part of the membranous labyrinth and are connected to the rest of the endolymphatic system where the utricular and saccular ducts unite. The human ED and ES runs through the vestibular aqueduct (VA), which is a bony J-shaped canal situated on the medial side of the vestibule and common crus (CC) (*Figure 1*).

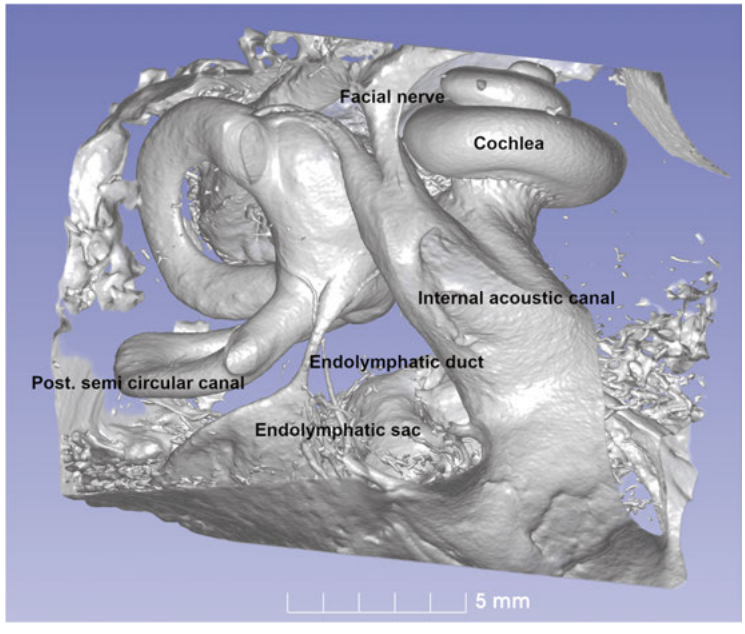

Figure 1. Micro-CT of left temporal bone, superior view.

The initial segment of the VA is narrow and fairly constant in shape and size [15,16], but gradually widens and opens up at the external aperture (or operculum) to the posterior cranial fossa. A pathologically widened VA can be found in LVAS, which often results in childhood deafness. Due to the three-dimensional J-shape of the VA, it is difficult to project and visualize these structures on computed tomography (CT) scans. Several proposals of optimal radiographic projections have been made.

The ES is divided into three parts on the basis of its morphological features: the proximal, intermediate, and distal parts [17,18]. The proximal part is located in the first part of the VA, while the intermediate part is located both in the VA and in the dura mater of the posterior cranial fossa. The distal part lies completely in the dural pouch on the posterior slope of the pyramid near the sigmoid sinus. The ED is fairly regular in size, while the ES shows spectacular anatomic variations, with surface areas differing by as much as 17 times [19]. The VA runs parallel to a bony channel termed the accessory canal (AC) (or para-vestibular canal). This channel contains the vein of the vestibular aqueduct (VVA), which drains blood from the vestibule to the sigmoid sinus [15,16]. Quite recently, a study of human temporal bones using light and electron microscopy revealed a peri-endolymphatic channel system surrounding the ED [20]; this system is assumed to be involved in endolymph absorption.

The human ES epithelium contains a heterogeneous population of cells and is arranged in a single layer with epithelial folds, polypous outgrowths, and

tubular formations [21]. Findings have suggested that the intermediate portion (also called the rugose part) may be more active in ion transport and fluid homeostasis [17,22]. Two distinguishable cell types, the mitochondria-rich cells (MRCs) and ribosome-rich cells (RRCs), exist in rats [14,22-24]. They correspond to the cyto-organelle-rich cells and filament-rich cells reported in mice [25], as well as light and dark cells in guinea pigs [17]. These cell types are thought to have different functions, such as fluid absorption and, possibly, secretion.

Historical and developmental aspects

The human VA was first described by the Neapolitan anatomist Domenico Cotugno in 1761[26]. He also described a small membranous sac in the dura adjacent to the VA and called the structure the "cavitas aquaeductus membranea." A detailed description of the membranous labyrinth was provided by Antonio Scarpa in 1789 [27]. He also recognized that the membranous labyrinth contained a separate fluid, endolymph. More than 100 years later, Siebenmann[28] presented careful drawings of inner ear casts and demonstrated the anatomy of the VA, the cochlear aqueduct, and their miniscule accessory canals. The first accurate description of the ES was in 1869, by Boettcher[29], and Hasse[30] introduced the term endolymphatic sac (*saccus endolymphaticus*) a few years later.

Expansion of the vestibule was discovered by Mondini in 1791[31], but it was not until almost 200 years later that Valvassori and Clemis[32] described an enlargement of the VA. The widening of the VA was associated with sensorineural hearing loss and was termed "large vestibular aqueduct syndrome," or LVAS.

In 1861, a French physician, Prosper Meniere, presented a paper in which he described a triad of symptoms compromising episodic vertigo, hearing loss, and tinnitus[33]. Previously, it had been thought that vertigo was a cerebral symptom, but he emphasized that vertigo could originate from damage to the inner ear. The condition he described now bears his name, but confusion regarding the clinical and pathological features of MD has prevailed for many years.

The inner ear has fascinated scientists through the years. The Swedish anatomist Gustaf Retzius (1842–1919) described the ear as the crown of creation and published his work on the inner ear's anatomy in different species in 1881 and 1884. Drawings of the inner ear by Retzius are amazingly accurate and are still fascinating[34].

From an evolutionary aspect, all vertebrates have an otic capsule with a fluid-filled membranous labyrinth containing sensory epithelia. The vestibular labyrinth contains a saccule, utricle, and semicircular canals and is used to detect gravity, balance, and movement. In all vertebrates, the semicircular canals and otholith organs are connected to an ED[35]. In some groups, like sharks

and ray fish (cartilaginous fishes), the duct ends in an open pore on the dorsal surface of the head (*Figure 2*). Small grains of sand can enter the inner ear through these pores and will, together with an endogenous material, form otoliths[36]. The French oto-surgeon Georges Portmann did experiments on sharks where he obliterated the ED opening. When he observed that the shark appeared to be unsteady, he drew the conclusion that the ED might be obstructed, causing increased pressure in the ear and thus vertigo in humans. He introduced ES surgery to relieve pressure 10 years before endolymphatic hydrops (EH) was described for the first time[37]. In bony fish and tetrapods, this external opening has been lost, and the ED can be seen to end in an ES (*Figure 2*). With the transition to living on land, instead of in water, and by that the necessity of detecting air-borne sounds, the cochlea eventually developed.

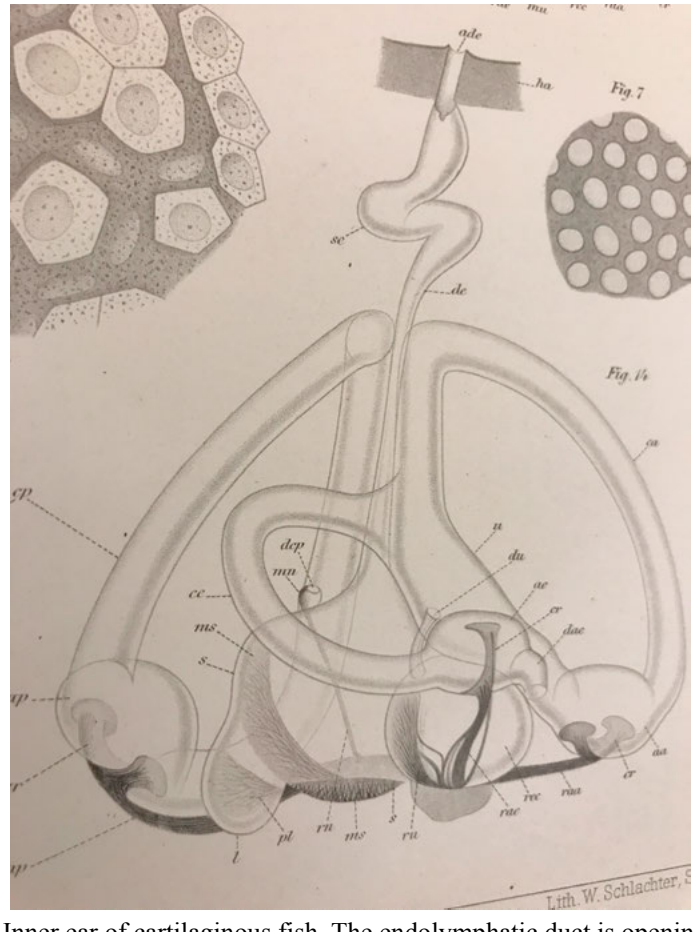

Figure 2. Inner ear of cartilaginous fish. The endolymphatic duct is opening up on the surface of the fish. There seems to be a plug or valvular structure in the opening. The semicircular canals can be seen. From Retzius 1884.

The earliest detailed description of the embryological development of the inner ear goes back to Streeter, in 1906[38]. Later studies have confirmed the results of this early work[39,40]. According to these studies, the differentiation between the ED and the cochlear duct (CD) starts at Carnegie stage CS17, and the semicircular ducts start to develop as a broad triangular elevation in the middle of the otic vesicle lateral to the ED at CS17. When the SCDs are starting to form at CS18–19, the ED is already visible as an elongated structure reaching further toward the posterior fossa. The ED is initially wide, but it gradually narrows, and the entire inner ear attains adult dimensions at midterm (*Figure 3*).

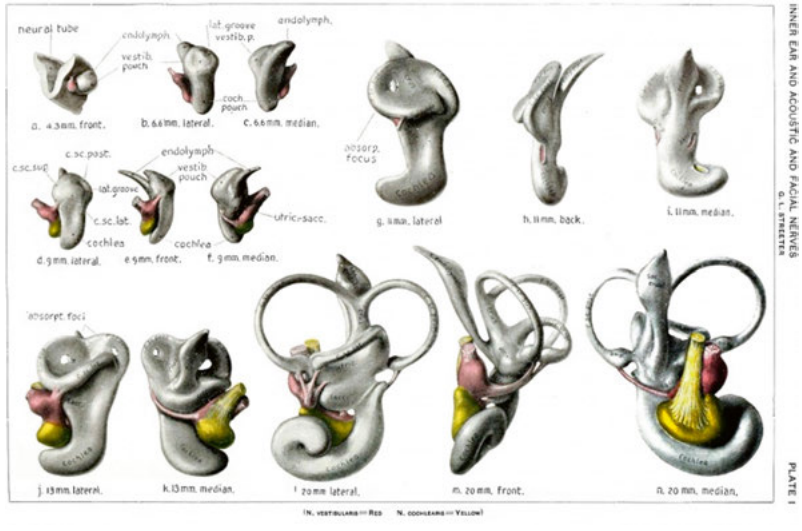

Figure 3. Development of the inner ear. From Streeter 1906.

While examining the collection of 324 inner ear casts belonging to the Uppsala archival temporal bone collection, one specimen was encountered that challenges what is known about inner ear development. In it, the VA first runs parallel to the CC, but then makes a bend, passing through the subarcuate fossa of the superior semicircular canal to continue its course to the posterior fossa (*Figure 4*). This suggests that the ES is formed early during inner ear development, and then followed by the differentiation and maturation of the semicircular canals. This type of malformation has not, to our knowledge, been observed before, and its functional consequences are unknown.

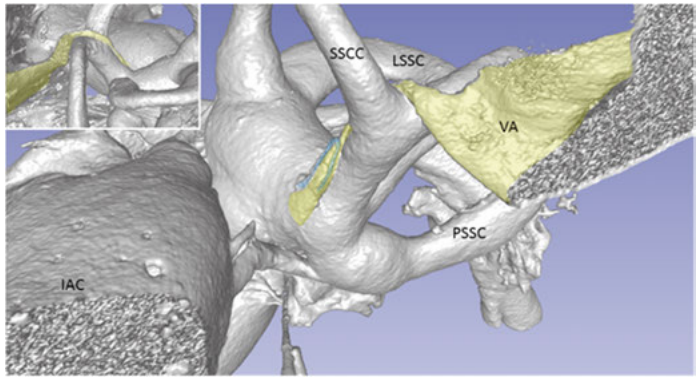

Figure 4. Micro-CT of inner ear cast showing the aberrant course of the VA (yellow). Median view. IAC, internal acoustic canal. SSCC, superior semicircular canal. LSSC, lateral semicircular canal. PSSC, posterior semicircular canal. VA, vestibular aqueduct.

Endolymph

The endolymph has a unique chemical composition suited to regulating the electrochemical impulses in hair cells. There are differences in resting potential, ion concentration, and pH between the endolymph in the scala media in the cochlea, the ES, and the vestibular organ. The resting potential in the ES is lower than in the cochlea, but higher than in the vestibular parts. In rodent cochlea, the potassium concentration is high (154.5 mM), and sodium concentration is low (0.23 mM), whereas in the ES, the potassium concentration is 11.6 mM, and the sodium concentration is 103.3 mM[41-45]. ES endolymph is more acidic (pH 6.7) than in the cochlea (pH 7.4). Many are of the opinion that there is a longitudinal "flow" of endolymph from the cochlea to the ES, but there is little substantial evidence to support this idea[12,46]. The production of endolymph from perilymph is made possible via selective ion transport over the Reissner's membrane[47]. It is still not completely understood where the endolymph is absorbed, but previous studies have indicated absorption in the ES or the ED[9,12,48].

The mechanical stimulation of hair cells opens up ion channels in the cell that, under the influence of an electro-chemical gradient, allows positively charged ions from the potassium-rich endolymph to enter. This creates a graded receptor potential in the inner hair cells that triggers the release of neurotransmitters that bind to the hearing nerve end terminus and initiates the action potential. The driving force for this mechanism is a field potential called the endocochlear potential (EP), as well as the high concentration of endolymph potassium. This potential is generated in the stria vascularis that secretes potassium into the endolymph, which is also recycling these ions. In the cochlea, the endolymph in scala media is separated from the perilymph in

scala vestibuli by the Reissner's membrane and from the perilymph in scala tympani by the basilar membrane.

Functions of the ED/ES

Many theories on the function of the ED and ES have been presented. They range over a wide field of suggested functions, such as the regulation of endolymph, pressure regulation, immune modulation, and more. Since most studies of the ED/ES are performed on animals, it might not be correct to apply those data to humans, since there are differences in anatomy and, perhaps, function among species.

Regulation of endolymph

Studies support the theory that the ED/ES is involved in endolymph homeostasis[47]. The major hypothesis is that the ED/ES is responsible for the endolymph uptake[8,9,49-52], but there are some studies supporting a secretory function[11,53,54]. More recent studies on the ES have revealed a number of proteins active in ion and water transport that are expressed in the ES epithelium[55-65]. This implies that the ED/ES is highly active in maintaining endolymphatic homeostasis. The exact mechanism for this is still not completely understood. The two main cell types in the ES are believed to have different functions. The MRCs are thought to absorb sodium and fluid from the ES lumen, whereas the RRCs might be responsible for secretion and immune activity[11].

Inner ear immunity

The inner ear was long thought to be devoid of immune incompetent cells. Several barriers exist to maintain the integrity of the membranous labyrinth[66], and it was believed that immune cells were not able to cross those barriers. Instead, the ES was thought to be the sole part of the inner ear responsible for an immune response[67-70]. More recently, though, immune cells have been found in other areas of the inner ear[71-74]. Two types of macrophages have been found to reside in the cochlea: cochlear macrophages, in the spiral ligament, and perivascular macrophage-like melanocytes, in the stria vascularis[75]. The macrophage-like melanocytes are believed to control the permeability of the tight junctions in the stria vascularis when immune responses are induced[75]. The precise role of the cochlear macrophages is not clear, but several studies suggest the phagocytosis of debris. The infiltration of IBA-1 positive cells in the cochlea after noise exposure has been shown[71,76-78], and experiments by Lang et al. suggested that the cells are bone marrow-derived[79]. A migration of macrophages into the cochlea in response to hair cells damaged by other agents, such as ototoxic drugs[80,81] and diphteria toxin-induced selective hair

cell degeneration[82], has also been shown. An immunologic route from the middle ear to the inner ear and the ES was proposed by Ikeda et al[83], and they also suggested that the ES was responsible for the local immune response for the inner ear. In guinea pigs that underwent unilateral ES obliteration, followed by sensitization and then challenge with the antigen, the cochlea showed a reduced amount of cellular infiltrate and antibody response in the ear where the ES had been obliterated[5], suggesting that the immune response in the inner ear is initiated in the ES. Histopathological findings of EH and vasculitis in the cochlea have been found in Cogan's syndrome, an autoimmune inner ear disease[84]. Magnetic resonance imaging (MRI) has also revealed EH in patients with primary immune-mediated inner ear disease[85].

Pressure regulation

Several authors have suggested that the ES plays a role in the regulation of intracochlear and intracranial pressure homeostasis, and in systemic and/or intracranial blood pressure[86-89]. The distal, extraosseous part of the ES lies close to the sigmoid sinus and the jugular bulb, which drains the venous blood from the brain. It is therefore possible that intracranial pressure changes could affect the ES. Vasopressin seems to have a regulatory effect on the aquaporin channels in the ES and increased serum vasopressin, and expression of the vasopressin receptor in the ES has been observed in EH[90,91]. Moller et al.[88] found the expression of natriuretic peptides in the human ES and hypothesized that the ES may regulate the inner ear's endolymphatic homeostasis and influence systemic and intracranial blood pressure.

Otologic disorders

Large Vestibular Aqueduct Syndrome (LVAS)

The association of congenital sensorineural hearing loss (SNHL) with an enlarged vestibular aqueduct was initially determined from histopathologic studies of inner ear malformations[92]. Radiologic detection of LVAS was reported in 1978 by Valvassori and Clemis, who described the enlargement of the VA in association with sensorineural hearing loss. It occurred mostly as a single trait, but could also be associated with other inner ear malformations such as Mondini's dysplasia. They called it "large vestibular aqueduct syndrome," or LVAS, and reported that a midpoint VA width of greater than 1.5 mm was to be considered abnormal[32]. This definition remains the most commonly used, although several other studies have suggested other criteria[93-95]. Due to the variable anatomy of the VA, it is difficult to project and visualize it using conventional computed tomography (CT). Several authors have presented rec-

ommendations for optimal radiological projections to visualize the VA. Proposals have been made in normal and pathological conditions and include axial (with or without reformats in the 45° oblique plane), lateral, and coronal projections.

The prevalence of LVAS ranges in different studies from 1.5% to 15% in pediatric populations with hearing loss[96,97]. Hearing loss in LVAS is often congenital, but can also be acquired during childhood and present as a sensorineural downward-fluctuating progressive high-frequency hearing loss[98] (*Figure 5*).

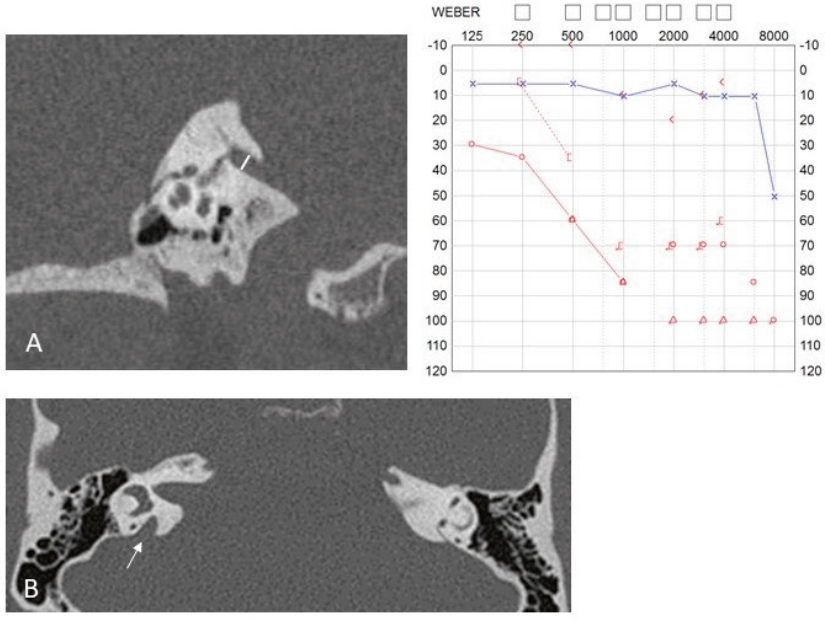

Figure 5. CT-scans (A, lateral and B, axial projections) and audiogram from an individual with unilateral (right-sided, arrow) severe hearing loss and LVAS.

Several genetic syndromes are associated with LVAS[99]. The most common is Pendred syndrome, which includes euthyroid goiter and LVAS[100]. Pendred syndrome is caused by a mutation in the SLC26A4 gene, which encodes the anion exchanger pendrin. Pendrin is present in the human ES and acts as a Cl-/HCO3 exchanger, playing an important role in maintaining the homeostasis of the endolymphatic fluid system[65,101]. Other syndromes linked to LVAS include distal renal tubular acidosis (ATP6B1 and ATP6N1B genes), Waardenburg's syndrome types 1 and 2 (PAX3 gene), X-linked congenital mixed deafness (POU3F4 gene), and branchio-oto-renal syndrome (EYA1 gene)[99]. There

have also been reports of familiar LVAS with no known mutation[102] or with unknown etiology.

In the case of mild to moderate hearing loss in LVAS, conventional hearing aids can be fitted. In congenital or acquired severe hearing loss or deafness, a cochlear implant (CI) may be indicated. CI surgery may be complicated by an intraoperative gusher, but it is considered to be a safe procedure with favorable outcomes[103].

Meniere's disease (MD)

MD is a chronic condition characterized by severe vertigo attacks, hearing loss, and tinnitus[104]. Population-based studies have reported a prevalence as high as 513 in 100,000[105]. The vertigo attacks can be devastating, and the severity is often associated with lowered quality of life[106]. EH has been revealed as the typical pathological finding in early human temporal bone studies[107,108]. EH is a distention of the endolymphatic space that most often occurs in the cochlear duct and the sacculus, but sometimes involves the semicircular canals and the utricle[109]. Recent studies have shown the possibility of visualizing EH in patients suffering from MD with MRI with gadolinium contrast[110,111]. The cause of the development of EH is still not clear.

Since Kimura showed that the obliteration of the ES and ED leads to EH, the ES has been assumed to be crucial for maintaining endolymphatic homeostasis[48]. There are several theories on the causes of MD and EH; these include a dysfunction of ES endolymph regulation by genetic or acquired causes[59,112], immunological disturbances/activation in the inner ear[68,113-116], hormonal disturbances[87], mechanical blockage by otoconia[117,118], and dysfunctional venous drainage[119,120].

Imaging techniques

The inner ear is difficult to study, due to its location within the densest bone in the body. Basically, there are two ways in which to study the inner ear's anatomy. In humans, histological studies can be made on cadavers or surgically removed inner ears, or the anatomy can be examined radiologically. Histology images with a conventional light microscope give us information down to the cellular level, but only of one thin slice at a time, which makes it difficult and time consuming to get 3D images. Conventional CT and MRI have a resolution of 0.5 mm, which is too coarse for evaluation of the micro-anatomy of the inner ear and its miniscule structures. Lately, techniques suitable for research in pre-clinical settings have been developed that have improved visualization. Super-resolution microscopy allows imaging down to the sub-cellular level on immunohistochemically prepared specimens, and novel radiological techniques give more detailed imaging of the temporal bones. The images

produced by micro-CT and SR-PCI can be fed into software that allows for 3D models to be made for quantitative analysis and visualization.

Confocal microscopy

This imaging technique works on the principle of blocking out disturbing out-of-focus light by using a spatial pinhole. This increases the optical resolution and contrast. By capturing multiple 2D images at different depths in the sample, a 3D image can be reconstructed.

Super-resolution structured illumination microscopy (SR-SIM)

Due to the diffraction of light, the resolution in conventional light microscopy is limited. Super-resolution microscopy is a term that gathers several techniques that allow images to be taken with a higher resolution than the one imposed by the diffraction limit. SIM is wide-field technique that works by using a patterned illumination (usually stripes) to excite the sample. The stripe position and orientation are moved a number of times, and the emitted fluorescence signal is recorded for each of these positions. The interaction of the fine-striped patterns of excitation and the sub-diffraction features in the sample emission creates Moiré patterns. All images from each position are collected and the final super-resolved image is reconstructed. The resolution for SR-SIM it is about 100 nm laterally and 250 nm axially. This allows subcellular structures to be visualized.

Micro-computed tomography (Micro-CT)

Micro-CT is an imaging technique similar to conventional CT. The sample is rotated and a series of X-ray images are taken. These images are then reproduced into cross-sectional images that can be further processed into 3D models. Micro-CT offers resolution down to the micrometer level but soft tissue reproduction is usually poor. It is only applicable in experimental situations due to the high-intensity irradiation accompanying the technique.

Synchrotron Radiation Phase Contrast Imaging (SR-PCI)

SR-PCI offers even greater resolution and better soft tissue contrast than micro-CT. A synchrotron is a large-scale particle accelerator that generates very bright, highly collimated light. The setup is similar to conventional radiography with an X-ray source, a sample and a detector. While CT imaging is absorption-contrast based, PCI relies on information concerning changes in the phase of an X-ray beam that passes through an object. The beam's phase shift caused by the sample is transformed into variations in intensity, which can be recorded by the detector.

Thesis aims

The general aims of the following studies were to investigate the VA, ED, and ES, focusing both on the molecular structure of the ES and the microanatomy of the ED in relation to LVAS and MD. Modern techniques for imaging and visualization have been used, in hopes of taking our knowledge of these miniscule inner ear structures further.

Paper I

The aims of this study were to find more accurate data concerning the normative values of the VA diameter. This would give us better possibilities for evaluating enlargement criteria at LVAS. For this, temporal bones from the Uppsala archival temporal bone collection and molds were used. The study also aimed to show the great anatomical variations of the human VA and to establish how these influence optimal radiographic inclinations and VA visualization

Paper II

In this paper, the focus was on investigating optimally fixed human ES removed during surgery using novel microscopic techniques, such as super resolution structured illumination microscopy (SR-SIM). This technique allows immunohistochemistry of molecular aggregates with a resolution of 100nm. The technique was recently used in the human cochlea after mild decalcification by my collaborators. The goal was to study the presence of immune cells in the ES with particular interest in the so-called IBA-1 cells, or macrophages, and to relate the findings to inner ear disease.

Paper III

In this study, the goal was to analyze the presence and distribution of Na/K-ATPase and associated ion channels, transporters and solute carriers, and hormone receptors (aldosterone). This information may have relevance to both

LVAS and Meniere's disease. The laboratory work for this study was done parallel to the work in Paper II using the same methods.

Paper IV

The goal of this paper was to analyze anatomical passages of endolymph outflow in the ED and peri-ductal capillary system using synchrotron phase contrast imaging in collaboration with researchers in Canada. A future intention was also to describe the findings in relation to inner ear disease especially MD.

Material and methods

Paper I and IV

Uppsala archival temporal bone collection

These two studies analyze specimens belonging to the Uppsala archival temporal bone collection. This museal collection from the 1970s consists of 113 human temporal bones from autopsies and 324 plastic and silicone molds made from the labyrinth. The ages and genders of the donors are unknown. The preparation and dissection of these autopsy specimens was done in the 1970s and 1980s by one of the co-authors (HR). Seventy-eight of the temporal bones were stained with 4% $OsmO^4$ solution, which was injected through the oval window and external aperture (EA) of the VA to mark the cavities of the labyrinth. The bones were then micro-dissected using dental drills. Several earlier publications have used data from this temporal bone collection[121,122]. More recently, these micro-dissected temporal bones have been analyzed further by other authors[123-127].

To create the silicone and plastic molds, the temporal bones were cleaned and then the cavities were filled with a polyester resin or silicone rubber material that was poured into the form in which the bones were placed. To facilitate the molding materials' entrance in the miniscule canals, the forms were placed into a low-pressure chamber. After hardening, the bone was dissolved with hydrochloric acid. Analyses of these molds have formed the basis for several earlier publications[128,129].

Paper I

Measurement procedure

In Paper I, both sets of temporal bones in the Uppsala archival temporal bone collection were used. Fifty micro-dissected temporal bones were randomly picked, and 32 were selected where the VA was clearly exposed. In addition, we randomly chose 20 plastic molds where the VA was visible and intact. All temporal bones and molds were photographed from different angles using a Zeiss V20 microscope. Measurements were made from the resulting photographs. In the micro-dissected temporal bones, we chose to measure the internal aperture, the sagittal width (height) of the EA, the total length of the VA,

the diameter of the proximal portion (at the most narrow point), and the thickness of the bone (the bony bridge or operculum at the EA). To show the variable anatomy of the VA, we also drew the outline of the VA in each specimen and digitized them. The molds were analyzed separately, and we focused on VA dimensions in the lateral and sagittal projections, such as the mid-distance between the common crus (CC) and EA of the VA. Mean values, ranges, and standard deviations (SD) were established and considered normative. For technical reasons, these mold specimens did not allow accurate estimation of the sagittal width (height) of the external aperture of the VA.

Paper II and III

Immunohistochemistry

In these two papers, immunohistochemistry was performed on five human ESs collected during trans-labyrinthine vestibular schwannoma surgery. The laboratory work for these studies was done simultaneously. The age, gender, and audiometric results of the donors were not known for ethical reasons. In the operating room, the specimens were immediately put in paraformaldehyde for fixation. Following decalcification with EDTA, the specimens were placed in sucrose overnight and then rapidly frozen. Embedded in Tissue-Tek (OCT Polysciences), they were sectioned at 8–10 μm using a Leica cryostat microtome. The frozen sections were collected onto glass slides and kept at -70°C or storage until immunohistochemistry was performed.

The sections, mounted on glass slides, were incubated overnight at 4°C with a solution containing the primary antibodies. After rinsing, secondary antibodies were added and slides were left to incubate at room temperature for 2 h in darkness. The sections were then counter-stained with DAPI to visualize the nuclei. The slides were covered with specific cover glasses. Antibody controls were performed, where the sections were incubated with BSA only, omitting the antibody. The control sections showed no visible staining in any part of the ES.

In Paper II, the focus was on studying the presence of immune cells in the ES, and antibodies targeting proteins that are specific for different immune cells and immune-mediators were chosen. The choice of antibodies was made to cover both the innate and adaptive immune systems (Table 1).

Table 1. *Antibodies used in Paper II*

Antibody	Type	Dilution	Host	Catalog no.	Producer
IBA-1	Polyclonal	1:100	Rabbit	PA5-27436	Thermo Fisher
CD68	Monoclonal	1:50	Mouse	NB100-683	Novus
MHCII	Monoclonal	1:100	Mouse	MA5-11966	Thermo Fisher
Collagen IV	Polyclonal	1:10	Goat	AB769	Millipore
Fractalkine	Monoclonal	1:50	Mouse	MAB3651-100	R&D Systems
TLR4	Oligoclonal	1:10	Rabbit	710185	Thermo Fisher
Vimentin	Monoclonal	1:50	Mouse	V5255	Sigma-Aldrich
CD11b	Monoclonal	1:50	Rabbit	AB52478	Abcam
CD4	Polyclonal	1:150	Goat	AF-379-NA	R&D Systems
CD8α	Monoclonal	1:100	Mouse	MAB1509	R&D Systems

In Paper III, the goal was to analyze the presence of ion channels, transporters, and solute carriers, as well as hormone receptors that could possibly mediate the activity of the channels and transporters. The focus was on Na/K-ATPase and its subunits and isoforms (Table 2). Cells expressing the Na/K-ATPase subunits β1 and α1 were analyzed numerically. Five sections that were stained for β1 and five sections stained for α1 were randomly chosen, and cells were counted manually.

Table 2. *Antibodies used in Paper III*

Antibody	Type	Dilution	Host	Catalog no.	Producer
Na/K-ATPase β1	Monoclonal	1:100	Mouse	MA3-930	Thermo Fisher
Na/K-ATPase β2	Polyclonal	1:50	Rabbit	ANP-012	Alomone Labs
Na/K-ATPase β3	Polyclonal	1:200	Rabbit	Abx111158	Abbexa
Na/K-ATPase α1'	Monoclonal	1:50	Mouse	SC21712	Santa Cruz Biotech.
Na/K-ATPase α1''	Monoclonal	1:50	Mouse	NB300-146	Novus
Na/K-ATPase α2	Polyclonal	1:100	Rabbit	OAAB03232	Aviva Systems Biol.
Na/K-ATPase α3	Monoclonal	1:50	Mouse	NB300-540	Novus
Carbonic anhydrase II	Polyclonal	1:50	Rabbit	HPA001550	Atlas Antibodies
NKCC2	Polyclonal	1:200	Rabbit	OABB01331	Aviva Systems Biol.
Mineralocorticoid receptor'	Monoclonal	1:50	Mouse	MA1-620	Thermo Fisher
Mineralocorticoid receptor''	Polyclonal	1:200	Rabbit	PA5-81527	Thermo Fisher
Vasopressin receptor type 2	Polyclonal	1:50	Rabbit	Abx133128	Abbexa
ENaC	Polyclonal	1:100	Rabbit	PA1-920A	Thermo Fisher
Aquaporin 2	Polyclonal	1:50	Rabbit	NB110-74682	Novus
Aquaporin 4	Polyclonal	1:50	Rabbit	AQP-004	Alomone Labs
Pendrin	Polyclonal	1.50	Rabbit	NBP1-60106	Novus
NKCC1	Polyclonal	1:100	Rabbit	AB59791	Abcam
NCC	Polyclonal	1:100	Rabbit	PA5-77816	Thermo Fisher

Imaging and photography

Two kinds of microscopes were used to investigate the stained sections. All sections were analyzed at our laboratory with laser confocal microscopy equipped with a three-channel laser emission system (Nikon TE2000). The optical scanning and processing of images was done with Nikon EZ-C1 (ver. 3.80) software and included the reconstruction of Z-stack images into 3D images. Selected slides with good quality were taken to the BioVis platform at

the Rudbeck laboratory for investigation with SR-SIM (Zeiss Elyra S.1 SIM system). Multicolor SR-SIM imaging was achieved with a four-channel laser setup. The images were processed with ZEN 2012 software (Zeiss), and 3D reconstruction was performed using Imaris 8.2 (Bitplane). Compared to confocal microscopy, the SR-SIM imaging offers better resolution and therefore the possibility to demonstrate proteins at the subcellular level. By combining the two methods, we were able to get images with an overview of their protein distribution and a more detailed localization of proteins in the cell.

Paper IV

Temporal bone preparation

In this study, temporal bones were studied using micro-CT or SR-PCI. The ones that underwent micro-CT were specimens belonging to the Uppsala temporal bone collection. Temporal bones that were subjected to SR-PCI were prepared and imaged by our co-authors at Western University, in London, Ontario, Canada. Five freshly frozen cadaveric temporal bones were used in this study. After thawing, the bones were cut to a sample of appropriately 40 x 60 mm. The bones were subsequently fixed in 4% formaldehyde and 3% glutaraldehyde in phosphate buffer for five days. After rinsing and dehydration in graded alcohol, the samples were transferred to the imaging facilities in motion-proof boxes.

Imaging

The analyses were made from micro-CT and SR-PCI images of temporal bones. All 113 temporal bones from the Uppsala temporal bone collection were subjected to micro-CT imaging, and five temporal bones imaged with SR-PCI were analyzed. The image data were imported to Slicer 4.6 and reconstructed into 3D digital models. This software allows for 3D models to be rendered transparent at cropping, and it is equipped with tools that make it possible to take measurements in 3D. The volume of the VA was measured on five randomly chosen specimens visualized with micro-CT. Volume assessments were done via segmentation by visually determined thresholding. The dimensions of the VA, the VVA, and the two main venous branches from the VVA were assessed. The two venous branches were designated as Branch 1, the branch situated inferior to the ED, and Branch 2, the branch situated superior to the ED.

Synchrotron radiation phase-contrast imaging

The five specimens were scanned using the BioMedical Imaging and Therapy 05ID-2 beamline at the Canadian Light Source Inc., in Saskatoon, Canada.

The setup was similar to conventional radiography, with a synchrotron beam produced by a superconducting wiggler source[130]. The beam was filtered by a monochromator, and the length of the beamline was 55 m from the source to the specimen. The image setup was made up by a sample stage and a charge-coupled device-based detector system, both positioned on a vibration isolation table. The distance between the sample and detector was 2 m. The resolution of the detector (an AA-60 beam monitor coupled with a C9300-124 camera, Hamamatsu Photonics, Shizuoka, Japan) was 12-bit, with an effective pixel size of 9 x 9 μm2. The image field of view was 4000 x 950 pixels, or 3.0 x 8.6 mm. In total, 3000 projections over a 180° rotation were acquired per view, and the exposure time was 100 ms per view. The final voxel size of the 3D image was 9 μm.

Micro-computed tomography

X-rays were generated in an X-ray source, transmitted through the temporal bone, and then recorded by an X-ray detector as a 2D image. The specimen was rotated, 360 degrees, a fraction of a degree at a time and more images were taken. These images were then computed into cross-sectional images that could be further processed into 3D models. The temporal bones in this study were scanned with a SkyScan 1176 micro-CT (Bruker, Kontich, Belgium).

Ethical considerations

Studies of the human inner ear are sparse. It is usually difficult to gain access to either fresh cadaveric or surgically removed inner ears, and in vivo studies on human material are practically impossible to perform. Research on the ES and ED has mainly been performed on animals, although the anatomy, and perhaps function, differ among species. With this in mind, it is important to study the human inner ear from both the anatomical and functional aspects in relation to human inner ear diseases. Our ethical permit gives us the opportunity to use inner ears, collected during surgery of cerebellopontine angle tumors, for research. This surgically removed tissue would otherwise have been wasted.

The studies of discarded human tissue in Papers II and III were approved by the local ethics committee (Etikprövningsnämnden Uppsala, no. 99398, 22/9 1999, cont, 2003, no. C254/4; no. C45/7 2007, Dnr. 2013/190), and patient consent was obtained.

The fresh frozen cadaveric temporal bones in Study IV were obtained with approval from the body bequeathal program at Western University, London, Ontario, Canada, in accordance with the Anatomy Act of Ontario and Western's Committee for Cadaveric Use in Research.

Results

Paper I

An analysis of the micro-dissected temporal bones showed that there were large variations in the anatomy of the VA. The shape of the VA was triangular, narrow proximally, and widening like a funnel distally (*Figure 6*). At the internal aperture, which formed a vertical crest medially, the VA was slightly wider. The VA was not in the same axial plane during its course through the temporal bones, and it curved laterally behind the posterior semicircular canal. The VA consisted of two parts, a proximal, which was fairly regular in shape, and a distal, which showed extensive variations in anatomy. The two parts were divided by the narrowest part of the VA, the isthmus, which was typically situated at the common crus. The distal part was large in the medio-lateral direction, but thin and leaf-like in the antero-posterior direction, as shown in *Figures 6 and 7*. In some of the specimens, the distal part of the VA displayed a notable "lateral recess." In two out of the 32 specimens, the isthmus was not discernible, seemingly not patent or obstructed. A small bony channel, the AC, was running parallel to the VA. It mostly ran inferior-medial to the VA, but in two specimens it was found laterally to the VA. In most cases, the AC seemed to open into the VA. Dimensions of the VA were assessed as shown in Table 3 and illustrated in *Figure 6*.

Table 3. *Dimensions of the VA in 32 micro-dissected temporal bones.*

	A	B	C	D	E	F
Mean measurement (mm)	2.29	5.72	6.50	0.30	1.49	0.54
Max	3.42	9.28	13.50	0.75	2.50	2.28
Min	1.34	3.23	3.32	0.13	0.42	0.20
SD	0.51	1.33	2.17	0.12	0.53	0.37

A indicates length of the VA from the internal aperture to the isthmus; *B*, length of the VA from the isthmus to the external aperture; *C*, width of the external aperture; *D*, diameter of the VA at the isthmus; *E*, thickness of the bony bridge, and *F*, height of the external aperture.

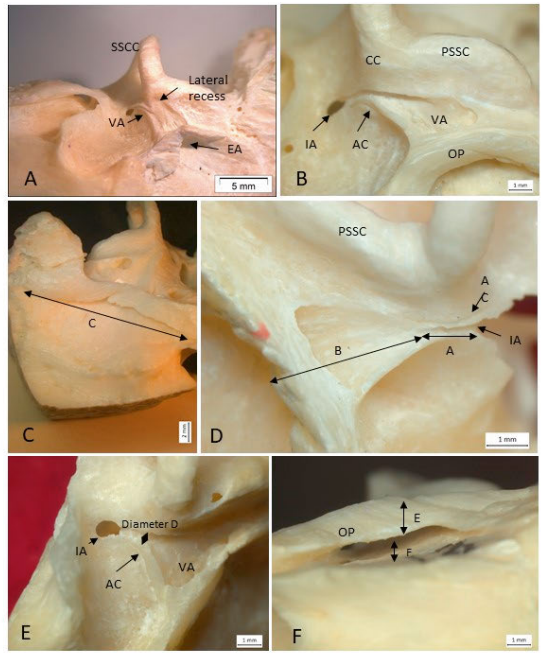

Figure 6. Micro-dissected human temporal bones showing the VA. **A**. Right temporal bone showing the VA, the super semicircular canal (SSCC), the external aperture (EA) and a lateral recess. **B**. Higher magnification of another specimen where the VA runs almost parallel to the posterior semicircular canal (PSSC). IA is the internal aperture. AC is the accessory canal. OP is the operculum.CC is the common crus. **C**. Left temporal bone viewed from posterior, showing the length of the external aperture, C. **D**. Left temporal bone where the accessory canal (AC) runs superior-laterally to the VA. Length of the VA from the IA to the isthmus (A) and from the isthmus to the EA (B) is shown. **E**. Right temporal bone showing an unusually wide VA. Diameter of the VA at the isthmus (D) is shown. **F**. View from inferior showing the EA with the operculum (OP). Thickness of the bony bridge (E) and height of the EA (F) are shown.

The plastic molds were also analyzed, and they clearly showed the anatomy of both the proximal and distal parts of the VA (*Figure 7*). Dimensions of the

VA at the mid-distance between the CC and the external aperture were assessed (diameter G). The mean dimension was 0.77 mm, min 0.54 mm, max 1.10, with SD 0.16 mm. In some specimens, the proximal part of the VA was surrounded by a vascular network and seemed to anastomose with the draining VVA. The results of the measurements in this study were compared to earlier studies.

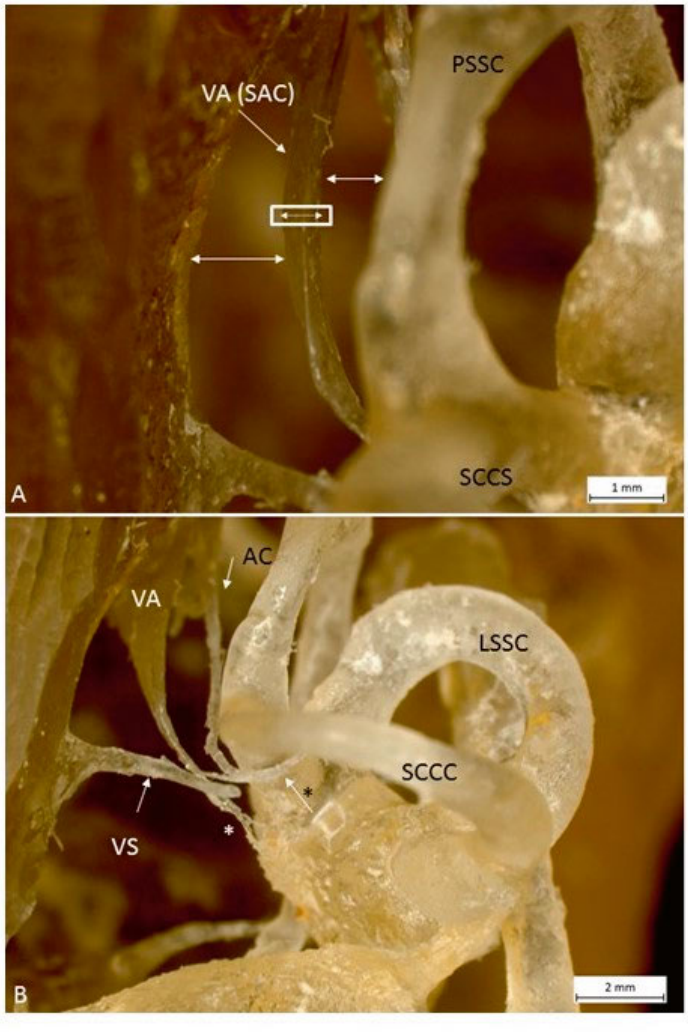

Figure 7. Plastic molds of the human inner ear. **A**. The VA housing the ES can be seen. The arrow in the box shows diameter G. **B**. The proximal portion of the VA is thin and hardly discernable. The accessory canal (AC) can be seen running parallel to the VA. VS; subarcuate vessels.

Paper II

Immunohistochemistry and imaging with SR-SIM and confocal microscopy revealed a multitude of immune cells in the ES.

IBA-1, which is regarded as a "pan-macrophage marker" expressed in all subpopulations of macrophages except alveolar macrophages (Kohler 2007), was expressed in a large number of cells residing both in the sub-epithelial tissue and the epithelium. The morphology of the cells was typical for macrophages, with branches and amoeba-like protuberances. Many cells in the perisaccular connective tissue expressed IBA-1. Some of these cells' morphology was similar to that of fibrocytes but distinguished from them by the IBA-1 expression in nuclei (*Figure 8*). Some IBA-1-expressing cells seemed to be part of the epithelia. The epithelial IBA-1 positive cells seemed to be both true epithelial cells and cells whose morphology were macrophage-like. In several places, IBA-1 positive cells showed signs of trans-epithelial migration and seemed to move from the epithelia to the ES lumen.

The epithelial cells expressed the chemokine fractalkine to various degrees (*Figure 8*).

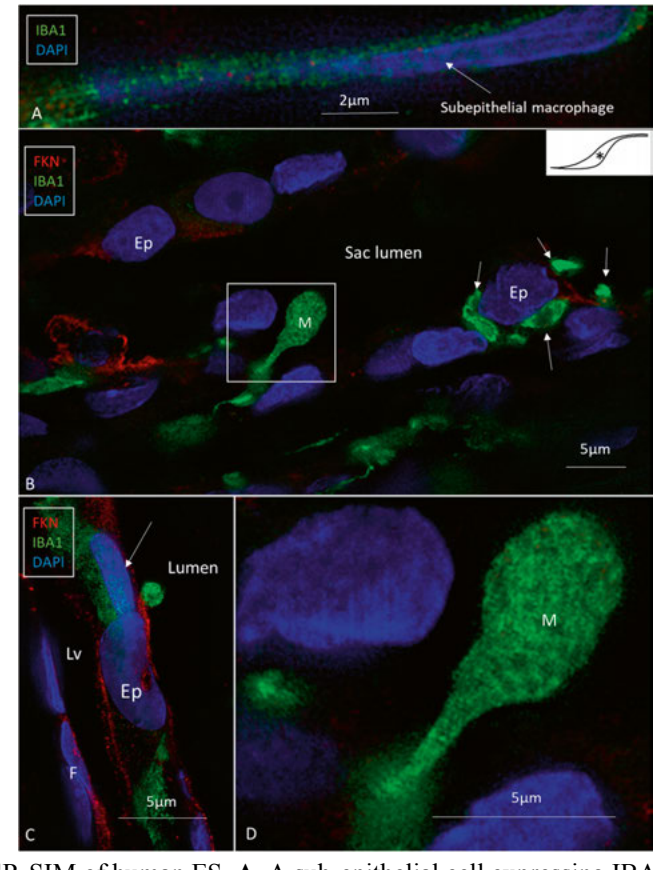

Figure 8. SR-SIM of human ES. **A**. A sub-epithelial cell expressing IBA-1. The nucleus is expressing IBA-1, distinguishing it from a fibrocyte. **B**. IBA-1 expressing cells with both sub-epithelial and epithelial location. One cell is seemingly being expelled into the ES lumen. Some epithelial cells express fractalkine. **C**. An IBA-1 expressing cell is situated in the epithelium. Fractalkine is expressed in epithelial and sub-epithelial cells. Lv, lymphatics. **D**. Framed area in B magnified. M, macrophage. *Shows location in ES.

MCH II and IBA-1 were co-expressed in many epithelial and non-epithelial cells. The co-expressing epithelial cells seemed to be both true epithelial cells and free intraepithelial cells. MHC II was mostly expressed in the apical membrane in the epithelial cells, some of these cells had large vesicles that opened up into the lumen of the ES (*Figure 9*). MHCII was also expressed in cells that did not express IBA-1 and those cells were seen interacting with IBA-1 positive cells.

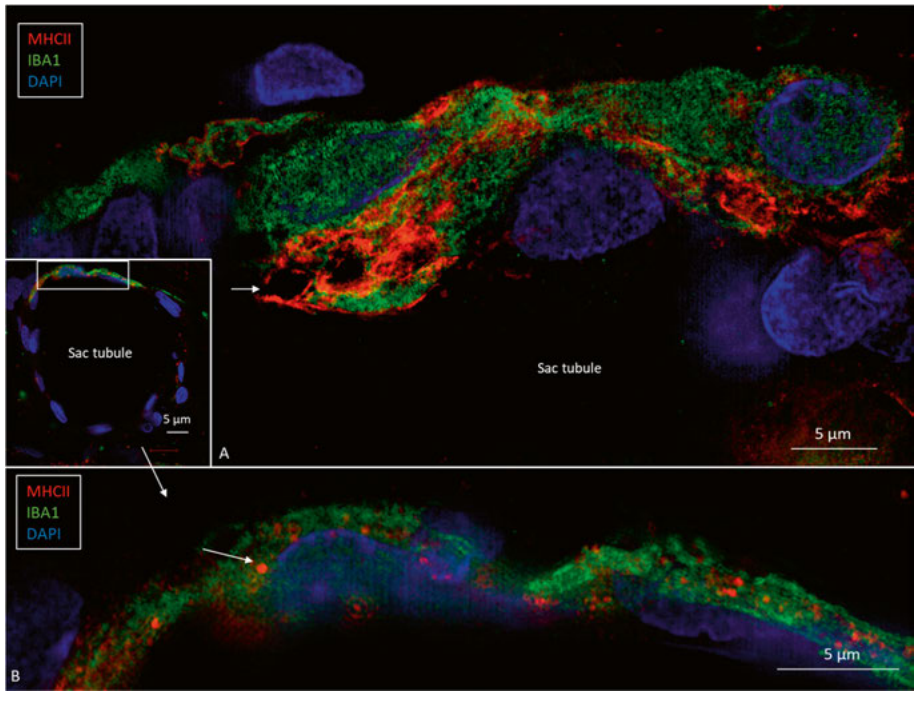

Figure 9. SR-SIM of human ES. **A**. IBA-1 expressing cells are located in the epithelium. The cells also express MHC II, especially in large vesicles (arrow). **B**. The framed area in the inset shown in higher magnification. Cell expressing both IBA-1 and MHC II.

Toll-like receptor 4 (TLR4) was expressed in the cytoplasm and cell membrane in sub-epithelial cells in the intermediate portion of the ES. Those cells did not co-express IBA-1.

CD68, a protein expressed by cells in the monocyte lineage, was expressed in a few sub-epithelial cells, occasionally together with IBA-1. CD11b, which is a protein expressed on the cell surface of many leukocytes including macrophages, was co-expressed with CD68 and MHCII on cells showing migrant behavior.

A sparse amount of CD4, as well as CD8-expressing cells, were seen in the ES, and physical interaction between CD4+ cells and IBA-1-expressing cells was noticed.

Paper III

Na/K-ATPase consists of four isoforms of the α subunit and three isoforms of the β subunit. Various Na/K-ATPase isozymes have different affinities for ions serving the specialized needs of different organs or tissues. SR-SIM and confocal microscopy of human ES showed staining of Na/K-ATPase subunits

α1, β1, and β3 in a large number of cells. There was no or weak staining for the subunits α2, α3, and β2.

Na/K-ATPase β1 was expressed in the basolateral cell membrane in cylindrical cells (*Figure 10*). No corresponding α subunit could be found. The β1-expressing cells were abundant, both in the intra- and extraosseous parts of the ES, but in the distal end of the ES, the expression was sparse. Some lower, cuboidal cells also expressed β1 in the basolateral membrane.

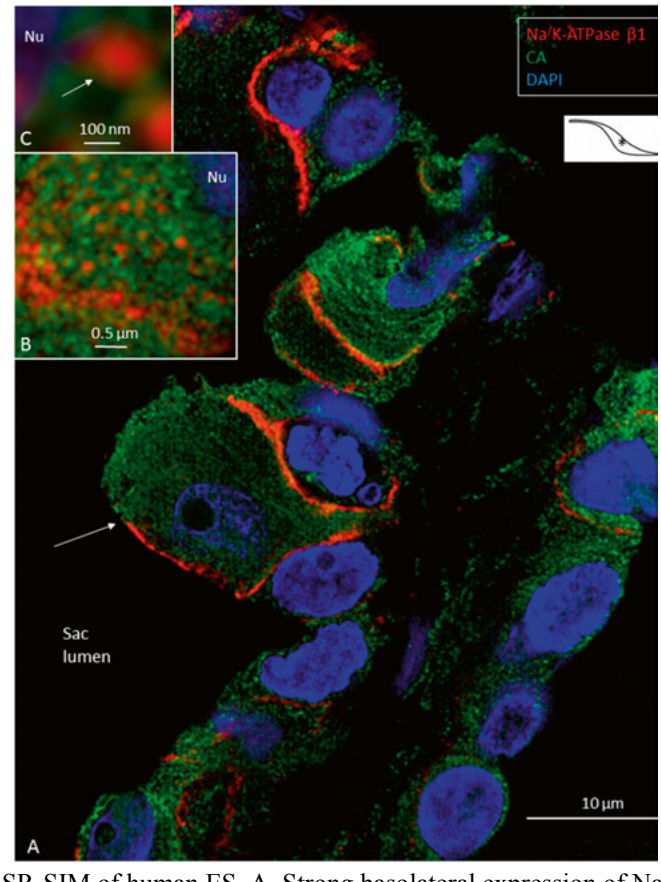

Figure 10. SR-SIM of human ES. A. Strong basolateral expression of Na/K-ATPase in cylindrical epithelial cells. Expression of CA. B and C. Higher magnification showing protein aggregates in the cytosol between the nucleus (Nu) and the plasma membrane. * showing level of sectioning.

The Na/K-ATPase subunits α1 and β3 were co-expressed in the apical cell membrane with "reversed polarity" in some cells. In a few cells, β3 was expressed without α1, adjacent to cells co-expressing α1 and β3 (*Figure 11*).

Carbonic anhydrase (CA) was expressed in cytoplasm in cells that also expressed Na/K-ATPase β1.

NKCC2 was expressed in β1-expressing cells as well as in cells with no Na/K-ATPase staining. NKCC1 was diffusely expressed in some epithelial cells.

Cubic and cylindrical cells expressed the amiloride-sensitive sodium channel ENaC and the thiazide-sensitive sodium channel NCC.

The mineralocorticoid receptor (MR) was expressed in many epithelial cells, both in the cytoplasm and in the cell nuclei. Pendrin and vasopressin receptor were diffusely expressed in some epithelial cells and aquaporin 2 and 4.

Quantification of Na/K-ATPase-expressing cells in the ES epithelia showed a basolateral expression of the β1 subunit in 78% of the cells, and 40% of the cells showed an apical expression of the α1 subunit.

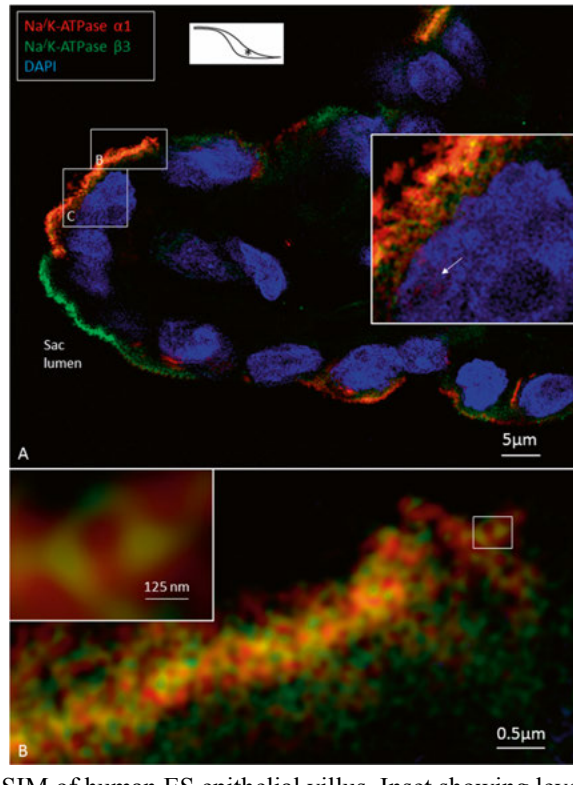

Figure 11. SR-SIM of human ES epithelial villus. Inset showing level of sectioning (*). **A**. Apical co-expression of Na/K-ATPase subunits α1 and β3 (framed areas B and C). Some cells are only expressing the β3 subunit. Inset showing framed areas B and C in higher magnification. The nucleus seems to contain α1 complexes while the cytoplasm stains positive for both isoforms. **B**. Strong expression of α1 and β3 subunits that seem to merge in the cell membrane. Inset in B shows the framed area in higher magnification.

Paper IV

Micro-CT of archival human temporal bones with 3D reconstruction revealed the bony VA and its accessory canals (*Figure 12*). The soft tissue could not be reproduced in the micro-CT images. The VA and AC showed extensive variations in anatomy. Two, or three bony channels could generally be seen originating at the internal aperture of the VA. These channels joined the VVA channel that emptied into the ES's extraosseous part. The volume of the VA as measured from the internal aperture to the isthmus was 0.32 µL (range, 0.24-0.43 µL).

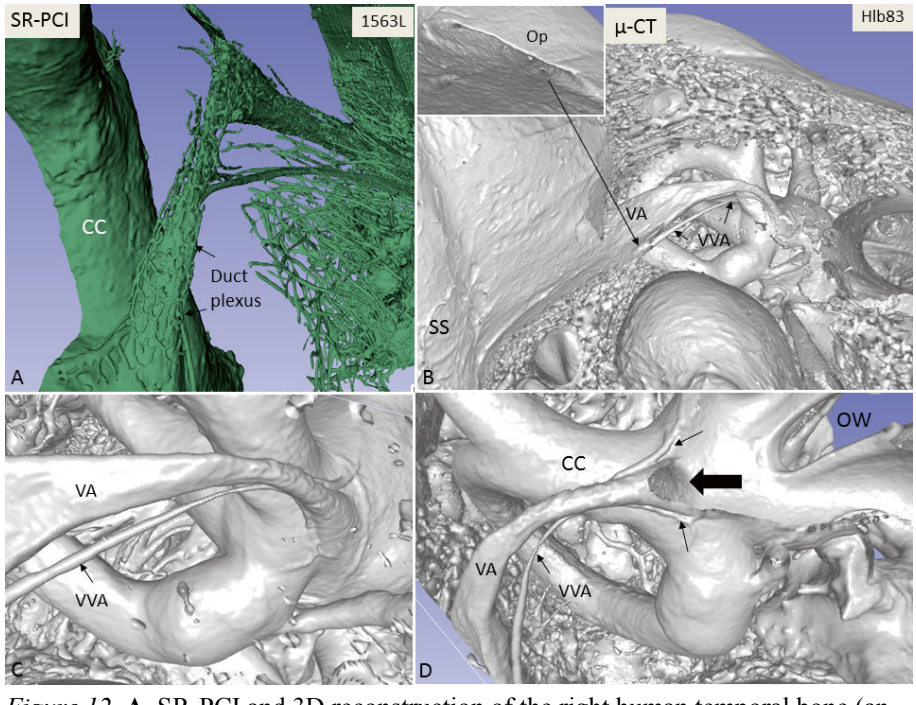

Figure 12. **A.** SR-PCI and 3D reconstruction of the right human temporal bone (anterior view). The extensive plexus surrounding the VA is shown. **B–D.** Micro-CT of left human temporal bone. **B.** The VA, VVA, and the posterior surface of the petrous pyramid. Inset shows the external aperture with the opening of the VVA (posterior view). **C.** Higher magnification of B. **D.** Micro-CT of the same specimen as B and C, showing the internal aperture of the VA (bold arrow) and surrounding veins (small arrow). CC, common crus; SS, sigmoid sinus; Op, operculum; VA, vestibular aqueduct; VVA, vein of the vestibular aqueduct; OW, oval window.

SR-PCI with 3D reconstruction could visualize both soft tissue and bone and identified a vast vascular plexus surrounding the VA (*Figures 12 and 13*). A scalar opacity mapping tool in the software was also used, making it possible to visualize small bone channels. Vessels could occasionally be seen in the bone channels. The plexus developed proximally and connected distally to

branches from the VVA, which further drained into the sigmoid sinus or the jugular bulb. These two venous branches that united to form the VVA correspond to the anterior and posterior branches of the VVA, as described by Nabeya[131]. It was obvious that the plexus in all specimens originated from around the VA and was not a portal system derived from the VVA. Less extensive plexa could also be seen surrounding the proximal and distal parts of the ES in one specimen, where the channel system was manually modeled (*Figure 13*). Measurements of the VA and venous branches are shown in Table 4.

Table 1. Dimensions of the VA and VVA (measurements in mm).

Specimen	VA diameter*	VA diameter long**	VA diameter short**	Diameter of Branch 1 of the VVA	Diameter of Branch 2 of the VVA
2R	0.26	0.81	0.37	0.11	0.10
1526R	0.17	0.75	0.30	0.13	0.05
1563L	0.18	0.54	0.26	0.06	0.07
1563R	0.14	0.83	0.30	0.06	0.09
1571x	0.16	0.88	0.54	0.05	0.05
Mean	0.18	0.76	0.35	0.08	0.07
SD	0.04	0.12	0.10	0.03	0.02

Branches 1 and 2 of the VVA were measured at a point as close to the VA as possible. VA; vestibular aqueduct. VVA; vein of the vestibular aqueduct. * diameter of the narrowest part of the VA (the isthmus). ** diameter at internal aperture measured at the longest and shortest distances.

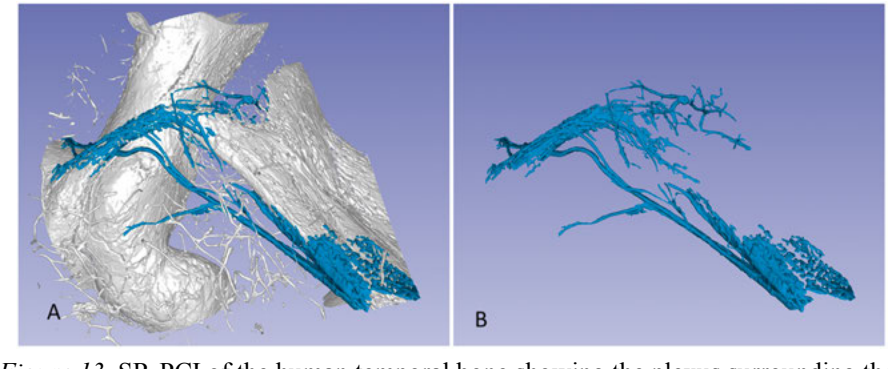

Figure 13. SR-PCI of the human temporal bone showing the plexus surrounding the VA as well as the proximal and distal part of the ES. The visualization was made after segmentation via manual threshold painting.

Discussion

For centuries, scientists have struggled to uncover the secrets of the VA, ED, and ES. Several different functions have been suggested. When trying to elucidate the mechanisms of these structures, it is important to have access to optimally preserved material. Most studies are performed on animals, and it may be difficult to extrapolate results from animal studies to humans. Since these structures are lodged inside the dense temporal bone, techniques have to be developed to gently decalcify the specimens while also preserving their structure and antigenicity.

The inner ear is difficult to study, since it not only lies sheltered in very dense bone, but also because it contains extremely vulnerable structures that are easily damaged during tissue-processing efforts. In fact, the human inner ear is likely the most difficult tissue to analyze in the human body.

As an audiological physician dealing with patients suffering from severe hearing loss and/or incapacitating vertigo on a daily basis, there is a desire to look inside and scrutinize the inner ear, to better understand the pathology and the pathogenesis of disease. Today, and even with the aid of greatly advancing imaging techniques, this is not possible, so we rely on different functional tests, and occasionally on genetic testing, radiology, and a patient's history, when making diagnoses.

In these projects, I had the opportunity to study the micro-anatomy and molecular structures of the secretive VA, ED, and ES on carefully preserved human inner ears. The Uppsala collection of temporal bones has been an invaluable asset, as has collaboration with the Canadian research team, who have shared their data and knowledge with us. A well-established collaboration with ENT surgeons has given us the opportunity to receive fresh human ESs from the operating theater for immunohistochemistry. Imaging facilities, especially the BioVis platform at Uppsala University, have provided us with state-of-the art technologies and further knowledge and assistance. In the quest to broaden the knowledge of the structure and functions of the inner ear, focus was on the VA, ED, and ES. What roles do these structures play in the development of inner ear disease? Can we further evaluate the micro-anatomy and receive more information about their function/s? This work is an attempt to further broaden our knowledge and serve as a platform for further research on these challenging and fascinating inner ear structures.

The enlarged Vestibular Aqueduct (VA)

In Paper I, the micro-anatomy of the VA was studied in light of earlier studies and results. We described the complicated 3D anatomy of the VA and the challenges in imaging it. Based on this, recommendations were given for criteria concerning enlargement and for imaging. But why is the VA enlarged, and what causes the resulting hearing impairment? Does the enlargement itself cause dysfunction in hearing, or is there something else that causes both enlargement and hearing impairment? LVAS is seen in Pendred syndrome, which is caused by a mutation in the SLC26A4 gene, encoding for the protein pendrin, an anion exchanger. In the inner ear, pendrin acts as an Cl-/HCO3 exchanger[65,101] and has been found in the ES. In Paper III, we stained for pendrin, but the results were inconsistent and not specific. This could be due to the method and lack of specificity of antibodies used in immunohistochemistry, or is it because pendrin is not expressed in adult humans' ES to any major degree? In mouse embryos, the lack of functional pendrin expression leads to the enlargement of scala media in the cochlea and the ED. The mouse embryo's ES starts to express pendrin at embryonic day (E) 11.5. In mouse embryos lacking pendrin, the enlargement of the endolymphatic spaces starts shortly after failed expression. Subsequently, the endolymph becomes more acidic and the EP drops[132]. The acidification affects all pH-sensitive mechanisms, and the enlargement may damage cell-to-cell communication, which could cause the hearing to decline at this stage or create an unstable hearing phenotype[133]. This could suggest that pendrin is important during the development of the inner ear, during the embryonic state. LVAS also occurs in other syndromes with mutations in genes encoding for proteins other than pendrin[99]. In these syndromes, the endolymph homeostasis could also be affected in the embryonic stage, thus causing the enlarged VA. LVAS can also be seen in cases where the etiology is unknown. It therefore seems likely that injuries to the inner ear of different etiologies and with different mechanisms could cause the enlarged endolymphatic spaces in embryos that evolve to LVAS. It is also possible that the initial injury that causes LVAS also deteriorates hearing. By contrast, it seems likely that an enlarged and distended endolymphatic space could be harmful in itself.

It is important to investigate the enlargement criteria for LVAS. It may be a marker for inner ear disease that also can be systemic. This may be of great importance for the clinician, since it may be associated with other symptoms. Furthermore, if cochlear implantation is planned, the surgeon could anticipate and prevent perilymph gusher. An accurate diagnosis of LVAS may also help to further understand some mechanisms causing inner ear disease.

Endolymphatic hydrops and Meniere's disease

According to the International Classification of Vestibular Disorders, ICVD, the diagnostic criteria for MD include two categories: definite MD and probable MD. These are symptomatically based, and a diagnosis of definite MD requires the observation of episodic vertigo attacks associated with low- to medium-frequency sensorineural hearing loss with fluctuating aural symptoms in the affected ear. Vertigo attacks should last between 20 minutes and 12 hours. Probable MD is defined by vertigo attacks lasting between 20 minutes and 24 hours and associated with aural symptoms[104]. There is no diagnostic biomarker for the disease, and the diagnostic criteria say nothing about the etiology or pathophysiology of MD, which is largely unknown. There are several differential diagnoses of MD, including vestibular migraine and autoimmune inner ear disease, which are sometimes difficult to distinguish from MD. Most scientists consider MD to be a multifactorial disease, with both genetic and environmental etiologic factors influencing the onset and course of the condition. MD is always associated with EH, as seen in histopathological temporal bone studies[107,108,134] and in MRI studies[110,111]. Even though EH can often be seen in MRI scans even in the contralateral asymptomatic ear, it seems likely that EH is necessary for the development of clinical definite MD and not a paraphenomenon. There have been numerous studies trying to address the cause of EH. Since Kimura et al.[10] found that the obstruction of the ED resulted in EH, the ES has been assumed to be crucial for maintaining endolymph homeostasis.

Na/K-ATPase plays a crucial role in ion and fluid transport of the inner ear, which contains systems responsible for the secretion of potassium into endolymph and the generation of the endocochlear potential. The α1β1 is the most common combination in epithelial cells for maintaining ion gradients[135]. In a recent study, the dense accretion of Na/K-ATPase αβ heterodimer complexes was exposed in the basolateral cell membrane of the marginal cells and outer sulcus in the human cochlea[136]. A strong basolateral distribution of Na/K-ATPase β1 isoforms, together with apical sodium-selective channels, suggests an active sodium uptake from the ES lumen with excretion into the subcellular space. These findings are consistent with earlier studies showing Na/K-ATPase in the adult ES[24,137-141]. ten Cate et al.[139] found α1 and β2 subunit isoforms and, to a lesser extent, the β1 subunit in guinea pig ES. They concluded that, since the combination is different from typical ion and fluid transporting tissues, such as those of the kidney and colon, it may reflect distinctive characteristics of inner ear Na/K-ATPase. Fina and Ryan[142] examined the mRNA-encoding isoforms and found only a weak expression of Na/K-ATPase, limited to α1, β1, and β2 in rats' ES. They suggested that the ES is not a major site for ion exchange. Indirect studies of epithelial transport by the endolymphatic sac potential, recorded after the injection of ouabain, were also unchanged[143,144].

In Paper III, antibodies against various isoforms of Na/K-ATPase and additional solute-transporting proteins were used, as these are believed to be essential for ion and fluid transport. A population of epithelial cells from the human ES strongly expressed Na/K-ATPase α1, β1, and β3 subunit isoforms in either the lateral/basolateral or apical plasma membrane domains. The β1 isoform was expressed in the lateral/basolateral plasma membranes in mostly large cylindrical cells, while β3 and α1 were both expressed with "reversed polarity" in the apical cell membrane in lower epithelial cells. The heterogeneous expression of Na/K-ATPase subunits substantiates earlier notions that the ES is a dynamic structure where epithelial cells show inverted epithelial transport. The findings in Paper III of epithelial cells that either express Na/K-ATPase in the basolateral membrane or in the apical cell membrane could support the view that the ES may both absorb and secrete endolymph (*Figure 14*). As mentioned above, such indications were obtained in earlier investigations by the group in Uppsala[11,145,146]. Rask-Andersen and Salt[145] performed combined physiological and anatomical studies of the ES and found evidence that the volume of fluid in the ES, and thereby the entire membranous labyrinth, may be regulated by a dynamic relationship between active secretion and the enzymatic degradation of a lumen-expanding substance associated with the intraluminal macrophages. It was not possible to establish the mechanisms behind these systems, or how they were related to ion and water movements across the epithelium of the ES.

The additional finding of mineralocorticoid receptors (MR) in the human ES epithelium suggests their involvement in fluid and ion regulation. Aldosterone, the main mineralocorticoid hormone, can, via the MR, regulate the activity of Na/K-ATPase[147]. Interestingly, there are more lines of evidence suggesting that aldosterone may regulate sodium transport in the ES. Previous studies found the epithelial sodium channel (ENaC) in cultured human ES epithelial cells and in rats' ES[56,148]. Furthermore, a sodium-chloride symporter (NCC) was also detected in rat ES epithelia[149]. Both ENaC and NCC are regulated by aldosterone. The findings in Paper III, of ENaC and NCC in the human ES epithelia, seem to support those earlier studies. Intra-tympanic steroids are widely used as treatment in MD and have been shown to be as effective as gentamycin in some studies[150]. The MR has equal affinity for glucocorticoids and mineralocorticoids, making it possible for glucocorticoids to regulate the ion transport in the ES[151].

Thus, the present findings give additional evidence about a dual absorptive/secretory function of the ES. Our results could suggest that the ES serves as a pressure regulator, monitoring and regulating inner ear fluid dynamics. Interestingly, it may also show that macrophages or immune cells existing in the ES may be involved in endolymph fluid regulation. These findings may shed new light on the etiology of endolymphatic hydrops and MD.

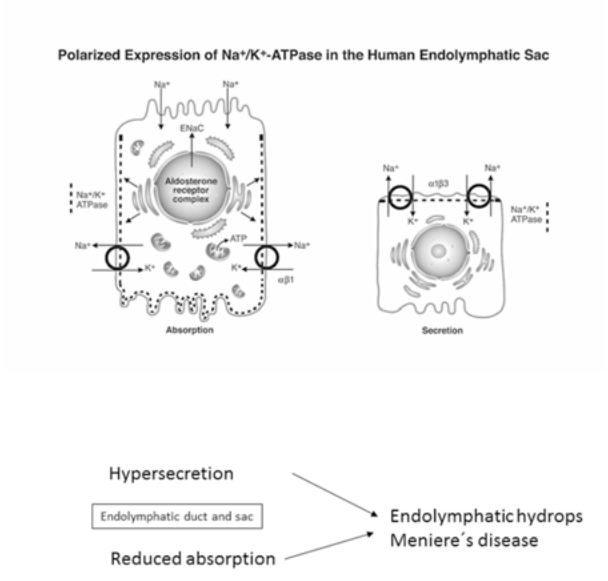

Figure 14. Suggested implication of a "reversed polarization" of Na/K-ATPase expression in the human ES.

The endolymphatic sac and immune function

The inner ear is protected by the so-called blood–labyrinth barrier, and it was once thought that immune cells could not penetrate this barrier[152-154]. It was later proposed that the blood–labyrinth barrier may consist of several barriers with different permeability for substances[66]. In one study from our group, perivascular IBA-1 positive cells, with no melanin, was found in the cochlear lateral wall [74]. Earlier studies have linked perivascular resident macrophage-like melanocytes to the regulation of the blood–labyrinth barrier[75]. A malfunction or absence of these cells could be harmful and reduce the EP, with accompanying hearing loss.

An important finding suggesting that the ES likely plays a key role in the immune defense of the inner ear was that ablation of the ES reduced the immune response in the cochlea after an antigen challenge in immunized animal models[155]. Furthermore, Möller et al. studied the gene expression of the humoral innate immune system in the human ES and found genes equivalent to that of the mucosa-associated lymphoid tissue (MALT)[6]. The expression was verified by immunohistochemistry, suggesting antigen recognition and processing for the initiation of immune responses. Multiple key elements of both the cellular and humoral innate immune-system were expressed, including toll-like receptors (TLRs 4 and 7, beta-defensin, and lactoferrin). According to Möller et al., the ES may provide defense not only against bacteria and

viruses but also against fungal infections of the inner ear. Altermatt et al. found immunostaining with antibodies against the CD4 and CD8 antigens and revealed a predominance of CD4+ T lymphocytes in the ES[156]. Findings of occasional lymphocytes in the cochlea support the theory of a "homing" of lymphocytes processed in the ES, as stipulated by Gloddek et al[157]. According to Yamada et al., the TLRs 2, 3, 4, and 9 are highly expressed in human ES fibroblasts and can produce cytokines and chemokines in response to the TLR ligands, thereby playing a role during the initiation of immune responses. The findings suggest that the human ES epithelium may trap and engulf intraluminal material reinforced by the TLR 4 signaling. SR-SIM also detected both CD4+ and CD8+ cells that interacted with macrophages. Danckwardt-Lilliestrom et al. showed ultrastructural evidence of cytotoxic lymphocyte activity in the ES epithelium of a patient with Meniere's disease[113]. It was associated with epithelial cell adhesion and degeneration, suggesting that toxic lymphocytes may be directed against a body's own ES cells with on-going autoimmune activity in some cases with this disease.

The findings in Paper II of large numbers of immune cells in the ES, corresponding to both the innate and adaptive immune systems, suggest that the ES plays a key role in the immune defense of the inner ear. In this paper, we speculate on an immune interaction between the ES and the rest of the inner ear. A large number of IBA-1 positive cells with different morphologies were found to reside in the ES; the cells populated surrounding connective tissue and the epithelium. Macrophages interacted with other cells, showed migrant behavior, and expressed immune cell markers, all of which suggests their active role in the innate and adaptive inner ear defense and tolerance. The processing of antigens and initiation of an immune response in the ES could be beneficial for the vulnerable hair cells in the cochlea, thereby avoiding a full-scale inflammatory response and the release of pro-inflammatory mediators and antimicrobial activity within the inner ear, which would be potentially harmful (*Figure 15*).

It has lately become clear that cochlear macrophages may have both beneficial and detrimental effects on the inner ear, perhaps reflecting different populations of macrophages. An intriguing interaction between the innate immune system and cochlear afferent neurons was described by Kaur et al.[82]. Damage to hair cells resulted in a chemokine signaling that protected spiral ganglion neurons. Neurons were found to be rescued following hair cell loss, but whether or not such mechanisms also prevail in humans is not known. The macrophages in the human ES, their capacity for immune processing, and their possible links to other cochlear and vestibular macrophages need further elucidation. We speculate that the immune response in the human ES may also protect the sensory organs. Thus, more studies are needed to fully understand the function of this hidden and intriguing activity in the human inner ear and its role in inner ear disease.

Several earlier findings have supported the theory that an immune injury may be important in the pathogenesis of MD[113,115,158]. Viral infection has been suggested as one cause of EH. Herpes simplex DNA has been found in the ES of MD patients[159], as have IgG antibodies against herpes simplex virus in the perilymph of MD patients[160]. Higher titres of IgG against adenovirus and varicella zoster virus compared to controls was also found[161]. Immunologic reactions or autoimmunity have been considered as causative factors in EH/MD. Autoimmune inner ear disease and MD seem to overlap clinically, and it is sometimes impossible to distinguish between the two. About one-third of MD cases have been reported to be of autoimmune origin[162,163]. Our findings support the theory that the ES is capable of initiating an inflammatory response that may be harmful to the inner ear and may present as MD. Damage can be due to the release of cytokines, or cross-reactions between inner ear antigens and virus/bacteria. In some cases, a genetic predisposition may prevail, with damage causing ionic imbalance leading to EH.

In a later review, we presented additional findings of macrophages in the ES, the cochlea, central and peripheral axons of the cochlear nerve, and the vestibular nerve trunk[164]. We speculated on a neuro-immunologic axis in the inner ear that could be important for the protection of the auditory nerve. Due to exposure to stressors and various harmful substances throughout life and an aging immune system, our bodies gradually up-regulate certain pro-inflammatory responses. This process has been named "inflammaging" and has been linked to several conditions such as atherosclerosis, type II diabetes, and chronic heart disease[165]. Inflammaging has also been linked to the neurodegenerative process in Alzheimer's disease[166]. A British cohort study showed that levels of inflammatory parameters increased with age and correlated with decreased hearing thresholds[167]. The inflammatory process initiated with aging might be one factor that influences age-related hearing loss and even the loss of auditory nerve fibers in the spiral lamina found in aged human cochleae[168].

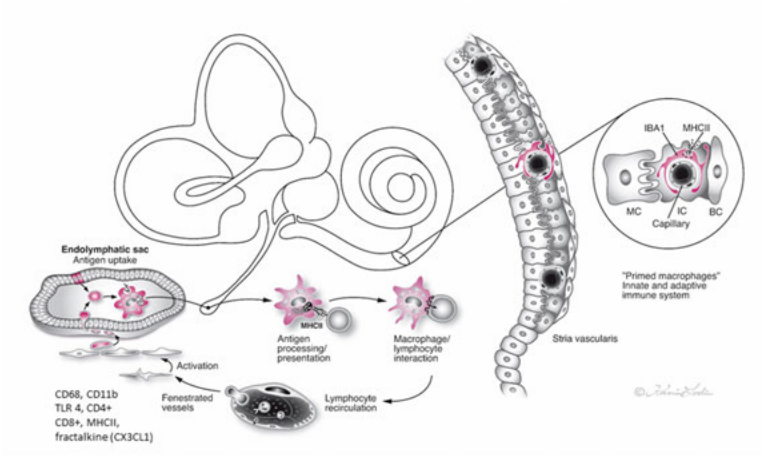

Figure 15. Proposed immune interaction between the ES and the inner ear. Waste material or antigens in the ES activates resident macrophages that can migrate into the sac lumen. After phagocytosis the macrophages migrate back into the subepithelial tissue for antigen presentation, thus initiating an immune response.

Synchrotron Imaging of the Human Endolymphatic Duct

In Paper IV, the main finding was a rich vascular network surrounding the ED. This plexus could be visualized three-dimensionally after reconstruction, which made it possible to establish its organization in more detail. It seemed to arise around the ED and not arise as a vascular portal system deriving from the vestibular venous drainage system. Instead, vessels derived directly from the ED and drained into the larger vein of the vestibular labyrinth, called the vein of the vestibular aqueduct (VVA). The vessels drained further into the sigmoid sinus.

The vascular plexus could represent the principal route for endolymph outflow, and we speculate that a malfunction of this associated system may result in EH. The situation recalls the channel/venous outflow system in the eye. The epithelial tight junctions in the ED have been shown to be more permeable than in the ES using freeze-fracturing technique and tracer studies[12,169]. As the potassium level in the endolymph is lower in the ES than in the cochlea, an outflow of potassium in the ED was previously suggested[12,170,171]. The "leaky" epithelium in the ED could allow for potassium and water to escape into the vascular plexus surrounding the ED for further transport to low-pressure veins (*Figure 16*).

The findings in Paper III revealed that a potent Na/K-ATPase pump is present in the ES. It may serve to direct fluid from the cochlea to the ES, representing an active "engine" responsible for the generation of a longitudinal endolymph flow from the labyrinth to the ED/ES. These findings are in line with the theory of a longitudinal flow of endolymph from the cochlea to the ES, based on classical experiments by Kimura, Guild, and Lundquist[9,50,172].

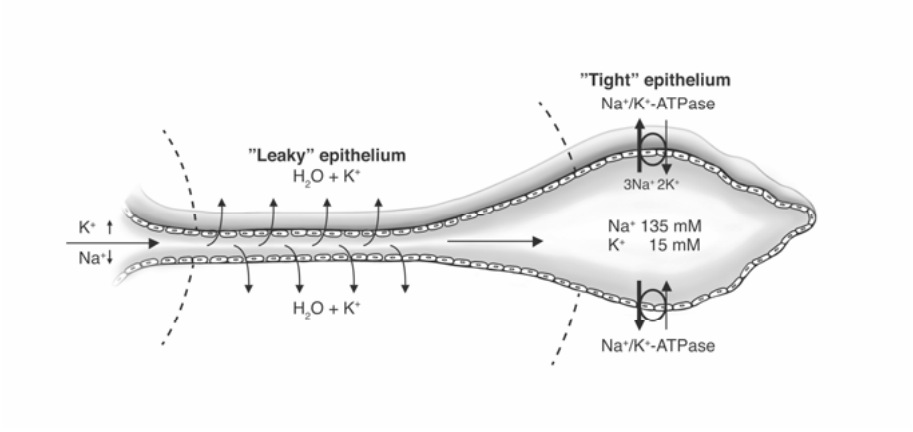

Figure 16. Schematic drawing showing proposed cellular functions in the human ED/ES. Active Na/K-ATPase ion pumps are localized in the wall of the ES. It may "pull" ions and fluid from the labyrinth to the ES and be responsible for the "longitudinal flow" of endolymph. Water and K^+ may escape proximally through the "leaky" epithelium in the ED to the surrounding lympho-venous channels

In 1871 Knapp pointed out the resemblance between ocular glaucoma and MD[173]. An impaired endolymph outflow could lead to EH, in analogy with ocular glaucoma. The principles of aqueous humor and endolymph outflow are diverse but there are some similarities. Both fluid systems contain a lympho-venous network with deeper connecting plexuses. In the eye most of the outflow resistance is located in the trabecular meshwork and collector channels which may be represented by the extra-cellular matrix and connective tissue resistance in the ED. Fluid influx into the Schlemm´s canal seems transcellular while in the ED, fluid transport seems para-cellular cross shallow epithelial tight junctions

Prostaglandin-F2a analogs are the first line therapy for reducing intra ocular pressure treat glaucoma and in one study, Latanoprost reduced vertigo and improved hearing in patients with MD in one study[174]. A reduced absorption of endolymph may be caused by an obstruction of the ED system[175]. This could be mechanical or instigated by inflammation leading to periductal fibrosis and density changes in the interstitial ground substance reducing lympho-capillary outflow. Our data in Paper IV infer that a patent VVA may be a requirement for ED drainage. An obstruction or anomalous course of the VVA

was described in MD[119,176] and a surgical procedure to decompress the ES/VVS to control vertigo and stabilize hearing in Meniere's disease has even been presented[177].

Hence, the findings made regarding the human ED and ES in the present thesis may hopefully lead to further understanding of the turnover and regulation of the inner ear fluids. This could also lead to improved treatment of some inner ear disorders such as MD.

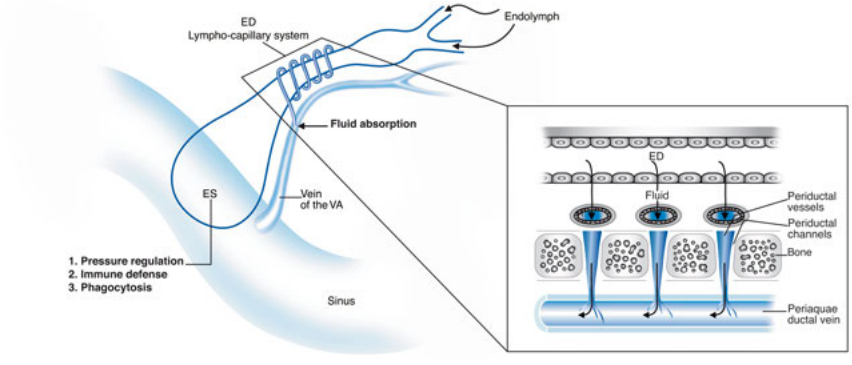

Figure 17. Drawing showing the principal drainage of endolymph in the human ED/ES. The ED is surrounded by vessels both in the periaqueductal and periductal spaces.

Methodological considerations

Papers I and IV share some methodological weaknesses that are linked to imaging and measurements. In both studies, only one person (the author) performed the measurements. All measurements were done with different computer software, but points of measurement were determined manually. In Paper IV, I measured the volume of the VA of five specimens based on micro-CT images with 3D reconstruction. Volume assessment was done via segmentation by visually determined thresholding, which makes comparisons between the specimens difficult to assess.

Both Papers I and II share the same kinds of strengths, namely the meticulously prepared and preserved temporal bones that make up the material for both studies. Shrinkage factor of the polyester resin casts was 0.6-1%, which is considered negligible with respect to the measurement procedure in this investigation.

In Papers II and III, one consideration was that the inner ear status could be altered from a normal material by the fact that all patients were suffering from vestibular schwannoma. The relatively small number of ESs could also be considered a weakness, but the results were very consistent among specimens. Immunohistochemistry has a number of potential pitfalls. Issues with fixation and sectioning, antibodies, and background staining can make results unreliable. The protocol used in these studies has been used in several earlier studies in our laboratory. All antibodies were used on several slides with sections from different specimens a number of times on different occasions. An obvious strength, and also a privilege, is that these studies were performed on human tissue, since most inner ear research has been performed on animals.

Conclusions

In this thesis, we analyzed the course and dimensions of the human vestibular aqueduct, which contains the endolymphatic duct and sac. Morphologic changes, such as obstruction and enlargement, have been linked to Meniere's disease and large vestibular aqueduct syndrome. By using the unique collection of human temporal bones in Uppsala, normative values were presented and criteria for enlargement given. The results may assist in evaluating these structures optimally via radiography and may form a basis for proper diagnosis. A lateral projection is recommended for optimal visualization via tomography.

This study also analyzed the human ES with super-resolution structured illumination microscopy. This technique allows, for the first time, the visualization of cells in this part of the human inner ear with a resolution down to 100 nm (immunohistochemistry and nanoscopy). High-resolution fluorescence microscopy showed MHCII-expressing macrophages and interaction with neighboring CD68, CD4, and TLR4 positive cells. The study shows that antigens reaching the ear may be trapped and processed by an immune cell machinery located in the ES. In this way, inflammatory activity may be evaded near the vulnerable sensory structures of the inner ear.

Similarly, the technique was used to reveal the expression of several isoforms of Na/K-ATPase and other solute-transporting proteins in the human ES. Na/K-ATPase β1 was strongly expressed in the basolateral cell membrane of epithelial cells, while Na/K-ATPase β3, together with the subunit α1, was expressed in the apical cell membrane in other types of cells. Based on those findings, we hypothesize that the ES is capable of both absorption and secretion and that the ES plays an active part in the dynamic regulation of inner ear fluid homeostasis.

Together with a Canadian research team, analysis of the human endolymphatic duct and sac using synchrotron phase-contrast imaging were made. Computer-based 3D reconstructions revealed a lympho-venous network surrounding the ED. This plexus originated from the ED and drained into collecting veins. It may constitute the principal outflow system for endolymph in man. Its importance for the generation of endolymphatic hydrops and Meniere's disease is discussed.

Future perspectives

The functions and involvement in inner ear disease of the human vestibular aqueduct, endolymphatic duct, and sac are still not fully understood. The ED and ES seem to fulfill several important functions for the inner ear. The present results have given further information about their role in endolymph drainage and immune activity. A further function not described in this work is endolymph pressure regulation and how it may be monitored in the human inner ear.

Future projects using immunohistochemistry (SR-SIM) may reveal the physiologic regulation of water-retaining proteins involved in fluid pressure regulation. Such studies must be combined with experimental and physiological research. The results presented here should be verified with other techniques, such as RNA sequencing and proteomics. Such studies are now also available in our laboratory.

The etiology behind MD remains an enigma. Is “finding the cause and cure of MD” as difficult as “finding the cure for cancer”? Meniere’s disease may not have one simple cause, but rather represent an outcome caused by several potential injuries to the inner ear. To find solutions for better treatment and a cure, we must broaden our understanding of the function of the inner ear and scrutinize different potential causes. A forthcoming possibility is local drug delivery to the inner ear via non-invasive or minimally invasive methods, something that research groups are presently evaluating.

One new and challenging field of research is inflammaging. To date, very little research has been devoted to examining the relationship between low-grade, chronic inflammation and age-related hearing loss. Further investigating the immunological state of the inner ear, and determining how it relates to ageing and hearing loss, may be a both challenging and intriguing future project.

Sammanfattning på svenska

Det mänskliga innerörat består i princip av tre olika delar, hörselorganet (snäckan), balansorganet samt den endolymfatiska gången och säcken. Hörsel- och balansorganens funktioner är väl studerade och kända medan den endolymfatiska gången och säckens funktioner fortfarande är oklara. Innerörat ligger väl skyddat i den så kallade benlabyrinten inuti tinningbenet. Benlabyrinten innehåller en hinnlabyrint som är fylld med endolymfa. Endolymfa är en unik vätska vars sammanställning exakt motsvarar de behov som innerörats hårceller har för att kunna omvandla ljud till nervsignaler. Den endolymfatiska gången och säcken förlöper i en benkanal, den vestibulära akvedukten, som sträcker sig medialt om snäcka och båggångar från balansapparaten där kanalerna från kristallorganen strålar samman. Kanalen förlöper ut på tinningbenets baksida där den öppnar sig mot bakre skallgropen. Den endolymfatiska säckens första del ligger i den vestibulära akvedukten omgiven av ben, medans den senare delen ligger i en ficka i hårda hjärnhinnan framför lillhjärnan.

Många olika teorier har presenterats vad gäller den endolymfatiska säcken och gångens funktioner. Tidigare forskning har påvisat immunologisk aktivitet samt reglering av endolymfans omsättning. Störningar i endolymfatiska gången och säcken har misstänkts ha en koppling till tillstånd som drabbar innerörat såsom Meniere's sjukdom och vidgning av vestibulära akvedukten (LVAS). Vid båda dessa tillstånd kan man se utbuktning av det endolymfatiska rummet och detta är kopplat till hörselnedsättning och yrsel.

Målet har varit att undersöka mikroanatomin och funktionella aspekter vad gäller vestibulära akvedukten, endolymfatiska gången och säcken. Genom att innerörat ligger så skyddat, inkapslat i hårt ben, så är det svårt att genomföra forskningsstudier på det. De flesta studier är gjorda på djur, men data som kommer från djurstudier kan inte säkert appliceras på människa. Genom möjligheten att utföra studier på mänskliga inneröron med nya och moderna tekniker har mer detaljerade studier kunnat göras i detta arbete.

I arbete I och IV användes mikrodissikerade tinningben samt avgjutningar av inneröron tillhörande Uppsalas temporalbens samling. I studie I detaljstuderades den endolymfatiska gången på dessa preparat med hjälp av mikroskop. Mätningar av denna gjordes för att få ett normalmaterial och därmed kunna fastställa när gången är sjukligt vidgad. I studien gavs rekommendationer vad gäller röntgenavbildning i olika tomografiska plan. Den endolymfatiska

gången har ett vindlande förlopp, och i vissa delar stora variationer och förändringar lätt kan misstolkas vid avbildning i bara ett tomografiskt plan.

I arbete II och III undersöktes den endolymfatiska säcken från människa med hjälp av immunohistokemi och högupplösande mikroskopi. I samband med kirurgi av tumör på balansnerven erhölls efter etiskt godkännande tillgång till endolymfatiska säckar som sedan fixerats, snittats och färgats med antikroppar mot proteiner av intresse.

I arbete II användes antikroppar mot proteiner som har anknytning till immunsystemet. Antikroppar valdes som speglar både det medfödda som det förvärvade immunsystemet. Med hjälp av konfokal samt högupplöst strukturerad illumineringsmikroskopi (high resolution structured illumination microscopy, SR-SIM) sågs en mängd makrofager i epitelial samt sub-epitelial vävnad, lymfocyter samt celler som uttryckte chemokinen fractalkine samt toll-like receptor. En modell för immunförvaret i inneröratupprättades. Hypotesen i studien är att endolymfatiska säcken kan fånga upp och identifiera främmande, skadliga agens såsom virus och bakterier och processa dem där samt initiera ett immunförsvar. Förprogrammerade lymfocyter kan sedan ta sig, via blodbanan, till andra delar av inneröratför att oskadliggöra skadliga agens.

I arbete III användes samma material och undersökningstekniker som i arbete II. Förekomst och uttryck för olika vätskereglerande jonkanaler, jonbytare och cellmembranskanaler som är genomsläppliga för vatten undersöktes. Fokus var framför allt på Na/K-ATPase och dess olika isoformer och subtyper. Många epitelceller i endolymfatiska säcken uttryckte Na/K-ATPase, subenhet β1 i basolaterala cellmembranet samt en att en annan typ av epitelcell uttryckte subenheterna α1 tillsammans med β3 apikalt i cellmembranet. Detta kan tyda på att endolymfatiska säcken har förmåga till både sekretion och absorption.

I arbete IV undersöktes vestibulära akvedukten, endolymfatiska säcken och gången på två olika typer av preparat samt med två metoder. Dels undersöktes plastgjutningar av inneröron tillhörande Uppsala temporalbenssamling med micro-CT men också inneröron med synkrotron strålning med faskontrast bildåtergivning (synchtrotron radiation phase contrast imaging, SR-PCI). Den endolymfatska gången omgavs av ett kärlplexa som såg ut att uppstå kring gången och som dränerade i den vestibulära akveduktens ven (vein of the vestibular aqueduct). Dessa plexa kan utgöra det huvudsakliga utflödet av endolymfa från den endolymfatiska gången.

Acknowledgements

This thesis is the result of the collaboration of many persons. Without you, this work would not have been possible to perform and I wish to express my sincere gratitude to you.

Special thanks to:

Professor Helge Rask-Andersen, my main supervisor, for your never-ending enthusiasm, support and generosity. Your vast knowledge of the inner ear combined with your skills and experience as a researcher have been invaluable during this project. Your energy and optimism are contagious, thanks for sharing them!

Professor Göran Laurell, my co-supervisor, for your wisdom and support. For our small-talks that has brought balance and structure during scientific ups and downs. For sharing your experience and help with financial support.

Co-author and collaborator Wei Liu. For your patient and friendly hands-on teaching in immunohistochemistry! For generously sharing your knowledge and experience. For interesting discussion about science and so much more during many hours in the lab or by the microscope.

Co-author and collaborator Hao Li. For your patient and kind support with all sorts of practical problems. It does not matter how big or small the issues are, you are always there!

Co-author and colleague, Niklas Danckwardt-Lilliestrom. For bringing me the best raw-material for my projects. For interesting discussions, both scientific and clinical.

Co-authors and collaborators, Sumit Agrawal and Hanif Ladak. For sharing the synchrotron images with us and for scientific support.

Karin Lodin. For beautiful illustrations.

Manochehr Amani, head of ENT department. For employing me and then allowing me to take time from clinical work to proceed with this work.

Elsa and Christine, my colleagues in the hearing and balance section at the ENT department. For your hard work and brilliant clinical skills with focus on helping our patients. For camaraderie, support and friendship. Thank you for patiently covering for my absence in the clinic!

Nadine, colleague and roommate. For inspiring discussions about science, clinical cases and everything else! You may know more about me than any other person by now.

Present and former colleagues and supervisors at the ENT clinic, Akademiska sjukhuset, Hörsel- och balanskliniken, Karolinska sjukhuset and ÖNH-kliniken, Östersunds sjukhus. You have all inspired me!

All audiologists, nurses, engineers and staff at the ENT department, Hörcentralen and the Cochlear Implant team for enjoyable cooperation and teamwork!

My friends, close and far away! Even though we might live far away from each other we always have a blast when we finally meet. I really enjoy your company while off-pist skiing, horseback riding, going to music festivals or just hanging around.

My family, close and extended:

My parents, Hildegun and Bengt Kämpfe. For unconditional love and support in any imaginable way.

My sister Helena and my brother Fredrik. For always being there. For all the fun times we have had together and will have in the future. For sharing many of my interests, especially music.

Margareta and Sven, my parents in law. For always supporting my family.

Simon, Selma and Sofia, my beautiful children. For being who you are and keeping my mind off research at times. I am proud of you. You are the best!

Jonas, my husband. For our family and for endless support. For lovingly being there during ups and downs.

And last, but not least. To Polly, my dog. The purest soul of all.

References

1 Rosenhall, U., Jonsson, R. & Soderlind, O. Self-assessed hearing problems in Sweden: a demographic study. *Audiology : official organ of the International Society of Audiology* **38**, 328-334 (1999).

2 Neuhauser, H. K. The epidemiology of dizziness and vertigo. *Handbook of clinical neurology* **137**, 67-82, doi:10.1016/b978-0-444-63437-5.00005-4 (2016).

3 Ten Voorde, M., van der Zaag-Loonen, H. J. & van Leeuwen, R. B. Dizziness impairs health-related quality of life. *Quality of life research : an international journal of quality of life aspects of treatment, care and rehabilitation* **21**, 961-966, doi:10.1007/s11136-011-0001-x (2012).

4 Rask-Andersen, H. & Stahle, J. Lymphocyte-macrophage activity in the endolymphatic sac. An ultrastructural study of the rugose endolymphatic sac in the guinea pig. *ORL; journal for oto-rhino-laryngology and its related specialties* **41**, 177-192, doi:10.1159/000275458 (1979).

5 Tomiyama, S. & Harris, J. P. The role of the endolymphatic sac in inner ear immunity. *Acta oto-laryngologica* **103**, 182-188 (1987).

6 Moller, M. N., Kirkeby, S., Vikesa, J., Nielsen, F. C. & Caye-Thomasen, P. Gene expression demonstrates an immunological capacity of the human endolymphatic sac. *The Laryngoscope* **125**, E269-275, doi:10.1002/lary.25242 (2015).

7 Salt, A. N. & DeMott, J. E. Ionic and potential changes of the endolymphatic sac induced by endolymph volume changes. *Hearing research* **149**, 46-54 (2000).

8 Secretan, J. P. De l'histologie normale du sac endolymphatique chez l'homme. *Acta Otolaryngol (Stockh.)* **32**, 119-163 (1944).

9 Guild, S. R. The circulation of the endolymph. *American journal of anatomy* **39**, 57-81 (1927).

10 Kimura, R., Schuknecht H. Membranous hydrops in the inner ear of the guinea pig after obliteration of the endolymphatic sac. *Pract Oto-Rhino-Laryngol.* **27**, 343-354 (1965).

11 Erwall, C., Jansson, B., Friberg, U. & Rask-Andersen, H. Subcellular changes in the endolymphatic sac after administration of hyperosmolar substances. *Hearing research* **35**, 109-118 (1988).

12 Rask-Andersen, H., Bredberg, G., Lyttkens, L. & Loof, G. The function of the endolymphatic duct--an experimental study using ionic lanthanum as a tracer: a preliminary report. *Annals of the New York Academy of Sciences* **374**, 11-19, doi:10.1111/j.1749-6632.1981.tb30855.x (1981).

13 Linder, B., Bostrom, M., Gerdin, B. & Rask-Andersen, H. In vitro growth of human endolymphatic sac cells: a transmission electron microscopic and immunohistochemical study in patients with vestibular schwannoma and

Meniere's disease. *Otology & neurotology : official publication of the American Otological Society, American Neurotology Society [and] European Academy of Otology and Neurotology* **22**, 938-943 (2001).
14 Rask-Andersen, H., Danckwardt-Lilliestrom, N., Linthicum, F. H., Jr. & House, W. F. Ultrastructural evidence of a merocrine secretion in the human endolymphatic sac. *The Annals of otology, rhinology, and laryngology* **100**, 148-156, doi:10.1177/000348949110000211 (1991).
15 Wilbrand, H. F., Rask-Andersen, H. & Gilstring, D. The vestibular aqueduct and the para-vestibular canal. An anatomic and roentgenologic investigation. *Acta radiologica: diagnosis* **15**, 337-355 (1974).
16 Ogura, Y. & Clemis, J. D. A study of the gross anatomy of the human vestibular aqueduct. *The Annals of otology, rhinology, and laryngology* **80**, 813-825, doi:10.1177/000348947108000608 (1971).
17 Lundquist, P. G., Kimura, R. & Wersaell, J. ULTRASTRUCTURAL ORGANIZATION OF THE EPITHELIAL LINING IN THE ENDOLYMPHATIC DUCT AND SAC IN THE GUINEA PIG. *Acta oto-laryngologica* **57**, 65-80 (1964).
18 Fukazawa, K., Sakagami, M., Matsunaga, T. & Fujita, H. Endocytotic activity of the free floating cells and epithelial cells in the endolymphatic sac: an electron microscopic study. *The Anatomical record* **230**, 425-433, doi:10.1002/ar.1092300315 (1991).
19 Friberg, U., Rask-Andersen, H. & Bagger-Sjoback, D. Human endolymphatic duct. An ultrastructural study. *Archives of otolaryngology (Chicago, Ill. : 1960)* **110**, 421-428 (1984).
20 Linthicum, F. H., Jr., Doherty, J., Webster, P. & Makarem, A. The periductal channels of the endolymphatic duct, hydrodynamic implications. *Otolaryngology--head and neck surgery : official journal of American Academy of Otolaryngology-Head and Neck Surgery* **150**, 441-447, doi:10.1177/0194599813516420 (2014).
21 Danckwardt-Lilliestrom, N., Friberg, U., Kinnefors, A. & Rask-Andersen, H. Ultrastructural analysis of 20 intraosseous endolymphatic sacs from patients with cerebello-pontine angle tumours. A surgically obtained control material for histopathological studies. *Auris, nasus, larynx* **27**, 311-321 (2000).
22 Dahlmann, A. & von During, M. The endolymphatic duct and sac of the rat: a histological, ultrastructural, and immunocytochemical investigation. *Cell and tissue research* **282**, 277-289 (1995).
23 Qvortrup, K., Rostgaard, J., Holstein-Rathlou, N. H. & Bretlau, P. The endolymphatic sac, a potential endocrine gland? *Acta oto-laryngologica* **119**, 194-199 (1999).
24 Peters, T. A., Kuijpers, W. & Curfs, J. H. Occurrence of NaK-ATPase isoforms during rat inner ear development and functional implications. *European archives of oto-rhino-laryngology : official journal of the European Federation of Oto-Rhino-Laryngological Societies (EUFOS) : affiliated with the German Society for Oto-Rhino-Laryngology - Head and Neck Surgery* **258**, 67-73 (2001).
25 Furuta, H., Mori, N., Fujita, M. & Sakai, S. Ultrastructure of the endolymphatic sac in the mouse. *Acta anatomica* **141**, 193-198 (1991).
26 Cotugno, D. *De aquaeductibus auris humanae internae.*, (Neapoli: Somoniana, 1760).
27 Scarpa, A. *Anatomicae disquisitiones de auditu et olfactu.*, (Pietro Galleati, 1789).

28 Siebenmann, F. Über die Injection der Knochenkanäle des Aquaeductus vestibuli et cochleae mit Wood´schem Metall. *Verhandl Naturf Gesellsch Basel* **8**, 672-684 (1889).
29 Boettcher, A. Über den Aqueductus vestibuli bei Katzen und Menschen. *Arch Anat Physiol* **36**, 372-380 (1869).
30 Hasse, C. Die Lymphbanen des inneren Ohres. *Anat Studien* **1**, 765 (1873).
31 Mondini, C. Caroli Mundini Anatomica surdi nati sectio. De bononiensi scientiarum et artium. . *Instituto atque Academica commentari.*, 419-431 (1791).
32 Valvassori, G. E. & Clemis, J. D. The large vestibular aqueduct syndrome. *The Laryngoscope* **88**, 723-728 (1978).
33 Menière, P. Mémoire sur des lésions de l'oreille interne donnant lieu à des symtomes de congestion cérébrale apoplectiforme. *Gaz Med Paris* **16**, 597-601 (1861).
34 Retzius, G. *Das Gehörorgan der Wirbelthiere: morphologisch-histologische Studien*. Vol. Vol II (Samson and Wallin, 1884).
35 Northcutt, R. G. *The evolutionary biology of hearing*. 21-48 (Springer, 1992).
36 Barber, V. C. & Emerson, C. J. Scanning electron microscopic observations on the inner ear of the skate, Raja ocellata. *Cell and tissue research* **205**, 199-215, doi:10.1007/bf00234680 (1980).
37 Portmann, G. The Saccus Endolymphaticus and an Operation for Draining for the Relief of Vertigo. *Proceedings of the Royal Society of Medicine* **20**, 1862-1867 (1927).
38 Streeter, G. On the development of the membranous labyrinth and the acoustic and facial nerve in the human embryo. *American journal of anatomy* **6**, 139-165 (1906).
39 Bast, T. H., Anson, B. J. & Gardner, W. D. The developmental course of the human auditory vesicle. *The Anatomical record* **99**, 55-74, doi:10.1002/ar.1090990105 (1947).
40 Arnold, W. H. & Lang, T. Development of the membranous labyrinth of human embryos and fetuses using computer aided 3D-reconstruction. *Annals of anatomy = Anatomischer Anzeiger : official organ of the Anatomische Gesellschaft* **183**, 61-66, doi:10.1016/s0940-9602(01)80014-5 (2001).
41 Konishi, T. & Hamrick, P. E. Ion transport in the cochlea of guinea pig. II. Chloride transport. *Acta oto-laryngologica* **86**, 176-184 (1978).
42 Bosher, S. K. The nature of the negative endocochlear potentials produced by anoxia and ethacrynic acid in the rat and guinea-pig. *The Journal of physiology* **293**, 329-345 (1979).
43 Amano, H., Orsulakova, A. & Morgenstern, C. Intracellular and extracellular ion content of the endolymphatic sac. *Archives of oto-rhino-laryngology* **237**, 273-277 (1983).
44 Mori, N., Ninoyu, O. & Morgenstern, C. Cation transport in the ampulla of the semicircular canal and in the endolymphatic sac. *Archives of oto-rhino-laryngology* **244**, 61-65 (1987).
45 Salt, A. N., Inamura, N., Thalmann, R. & Vora, A. Calcium gradients in inner ear endolymph. *American journal of otolaryngology* **10**, 371-375 (1989).
46 Salt, A. N. & DeMott, J. Longitudinal endolymph flow associated with acute volume increase in the guinea pig cochlea. *Hearing research* **107**, 29-40 (1997).
47 Sterkers, O., Ferrary, E. & Amiel, C. Production of inner ear fluids. *Physiological reviews* **68**, 1083-1128, doi:10.1152/physrev.1988.68.4.1083 (1988).

48 Kimura, R. S. Experimental blockage of the endolymphatic duct and sac and its effect on the inner ear of the guinea pig. A study on endolymphatic hydrops. *The Annals of otology, rhinology, and laryngology* **76**, 664-687, doi:10.1177/000348946707600311 (1967).
49 Siirala, U. Ueber den Bau und die Funktion des Ductus und Saccus endolymphaticus bei alten Menschen. *Z Anat Entwicklungsgesch.* **3**, 246-265 (1941).
50 Lundquist, P. G., Kimura, R. & Wersaell, J. EXPERIMENTS IN ENDOLYMPH CIRCULATION. *Acta oto-laryngologica. Supplementum* **188**, Suppl 188:198+ (1964).
51 Zechner, G. & Altmann, F. Histological studies on the human endolymphatic duct and sac. *Practica oto-rhino-laryngologica* **31**, 65-83 (1969).
52 Friberg, U., Bagger-Sjoback, D. & Rask-Andersen, H. The lateral intercellular spaces in the endolymphatic sac. A pathway for fluid transport? *Acta oto-laryngologica. Supplementum* **426**, 1-17 (1985).
53 Erwall, C., Takumida, M., Bagger-Sjoback, D., Rask-Andersen, H. & Wroblewski, J. Uptake of radioactive sulphur in the endolymphatic sac. An autoradiographic study. *Acta oto-laryngologica* **107**, 63-70 (1989).
54 Jansson, B., Friberg, U. & Rask-Andersen, H. Effects of glycerol on the endolymphatic sac. A time sequence study. *ORL; journal for oto-rhino-laryngology and its related specialties* **54**, 201-210, doi:10.1159/000276299 (1992).
55 Mori, N. & Wu, D. Low-amiloride-affinity Na+ channel in the epithelial cells isolated from the endolymphatic sac of guinea-pigs. *Pflugers Archiv : European journal of physiology* **433**, 58-64 (1996).
56 Kim, S. H., Park, H. Y., Choi, H. S., Chung, H. P. & Choi, J. Y. Functional and molecular expression of epithelial sodium channels in cultured human endolymphatic sac epithelial cells. *Otology & neurotology : official publication of the American Otological Society, American Neurotology Society [and] European Academy of Otology and Neurotology* **30**, 529-534, doi:10.1097/MAO.0b013e31819a8e0e (2009).
57 Miyashita, T., Tatsumi, H., Furuta, H., Mori, N. & Sokabe, M. Calcium-sensitive nonselective cation channel identified in the epithelial cells isolated from the endolymphatic sac of guinea pigs. *The Journal of membrane biology* **182**, 113-122 (2001).
58 Kakigi, A., Nishimura, M., Takeda, T., Taguchi, D. & Nishioka, R. Expression of aquaporin1, 3, and 4, NKCC1, and NKCC2 in the human endolymphatic sac. *Auris, nasus, larynx* **36**, 135-139, doi:10.1016/j.anl.2008.04.012 (2009).
59 Kumagami, H. *et al.* Expression of the osmotically responsive cationic channel TRPV4 in the endolymphatic sac. *Audiology & neuro-otology* **14**, 190-197, doi:10.1159/000180290 (2009).
60 Kim, S. H. *et al.* Electrogenic transport and K(+) ion channel expression by the human endolymphatic sac epithelium. *Scientific reports* **5**, 18110, doi:10.1038/srep18110 (2015).
61 Mizukoshi, F., Bagger-Sjoback, D., Rask-Andersen, H. & Wersall, J. Cytochemical localization of Na-K ATPase in the guinea pig endolymphatic sac. *Acta oto-laryngologica* **105**, 202-208 (1988).
62 Stankovic, K. M., Brown, D., Alper, S. L. & Adams, J. C. Localization of pH regulating proteins H+ATPase and Cl-/HCO3- exchanger in the guinea pig inner ear. *Hearing research* **114**, 21-34, doi:10.1016/s0378-5955(97)00072-5 (1997).

63 Dou, H. *et al.* Co-expression of pendrin, vacuolar H+-ATPase alpha4-subunit and carbonic anhydrase II in epithelial cells of the murine endolymphatic sac. *The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society* **52**, 1377-1384, doi:10.1177/002215540405201014 (2004).
64 Takumida, M., Bagger-Sjoback, D. & Rask-Andersen, H. Ultrastructural localization of carbonic anhydrase and its possible role in the endolymphatic sac. *ORL; journal for oto-rhino-laryngology and its related specialties* **50**, 170-175, doi:10.1159/000275986 (1988).
65 Moller, M. N., Kirkeby, S., Vikesa, J., Nielsen, F. C. & Caye-Thomasen, P. Gene expression in the human endolymphatic sac: the solute carrier molecules in endolymphatic fluid homeostasis. *Otology & neurotology : official publication of the American Otological Society, American Neurotology Society [and] European Academy of Otology and Neurotology* **36**, 915-922, doi:10.1097/mao.0000000000000669 (2015).
66 Sun, W. & Wang, W. Advances in research on labyrinth membranous barriers. *Journal of otology* **10**, 99-104, doi:10.1016/j.joto.2015.11.003 (2015).
67 Rask-Andersen, H. & Stahle, J. Immunodefence of the inner ear? Lymphocyte-macrophage interaction in the endolymphatic sac. *Acta oto-laryngologica* **89**, 283-294 (1980).
68 Rask-Andersen, H., Danckwardt-Lilliestrom, N., Friberg, U. & House, W. Lymphocyte-macrophage activity in the human endolymphatic sac. *Acta oto-laryngologica. Supplementum* **485**, 15-17 (1991).
69 Harris, J. P. & Ryan, A. F. Immunobiology of the inner ear. *American journal of otolaryngology* **5**, 418-425 (1984).
70 Harris, J. P., Heydt, J., Keithley, E. M. & Chen, M. C. Immunopathology of the inner ear: an update. *Annals of the New York Academy of Sciences* **830**, 166-178 (1997).
71 Hirose, K., Discolo, C. M., Keasler, J. R. & Ransohoff, R. Mononuclear phagocytes migrate into the murine cochlea after acoustic trauma. *The Journal of comparative neurology* **489**, 180-194, doi:10.1002/cne.20619 (2005).
72 Okano, T. *et al.* Bone marrow-derived cells expressing Iba1 are constitutively present as resident tissue macrophages in the mouse cochlea. *Journal of neuroscience research* **86**, 1758-1767, doi:10.1002/jnr.21625 (2008).
73 Warchol, M. E., Schwendener, R. A. & Hirose, K. Depletion of resident macrophages does not alter sensory regeneration in the avian cochlea. *PloS one* **7**, e51574, doi:10.1371/journal.pone.0051574 (2012).
74 Liu, W., Molnar, M., Garnham, C., Benav, H. & Rask-Andersen, H. Macrophages in the Human Cochlea: Saviors or Predators-A Study Using Super-Resolution Immunohistochemistry. *Frontiers in immunology* **9**, 223, doi:10.3389/fimmu.2018.00223 (2018).
75 Zhang, W. *et al.* Perivascular-resident macrophage-like melanocytes in the inner ear are essential for the integrity of the intrastrial fluid-blood barrier. *Proceedings of the National Academy of Sciences of the United States of America* **109**, 10388-10393, doi:10.1073/pnas.1205210109 (2012).
76 Tornabene, S. V., Sato, K., Pham, L., Billings, P. & Keithley, E. M. Immune cell recruitment following acoustic trauma. *Hearing research* **222**, 115-124, doi:10.1016/j.heares.2006.09.004 (2006).
77 Fredelius, L. & Rask-Andersen, H. The role of macrophages in the disposal of degeneration products within the organ of corti after acoustic overstimulation. *Acta oto-laryngologica* **109**, 76-82 (1990).

78 Fredelius, L. Time sequence of degeneration pattern of the organ of Corti after acoustic overstimulation. A transmission electron microscopy study. *Acta oto-laryngologica* **106**, 373-385 (1988).
79 Lang, H. *et al.* Contribution of bone marrow hematopoietic stem cells to adult mouse inner ear: mesenchymal cells and fibrocytes. *The Journal of comparative neurology* **496**, 187-201, doi:10.1002/cne.20929 (2006).
80 Sato, E., Shick, H. E., Ransohoff, R. M. & Hirose, K. Expression of fractalkine receptor CX3CR1 on cochlear macrophages influences survival of hair cells following ototoxic injury. *Journal of the Association for Research in Otolaryngology : JARO* **11**, 223-234, doi:10.1007/s10162-009-0198-3 (2010).
81 Ladrech, S., Wang, J., Simonneau, L., Puel, J. L. & Lenoir, M. Macrophage contribution to the response of the rat organ of Corti to amikacin. *Journal of neuroscience research* **85**, 1970-1979, doi:10.1002/jnr.21335 (2007).
82 Kaur, T. *et al.* Fractalkine Signaling Regulates Macrophage Recruitment into the Cochlea and Promotes the Survival of Spiral Ganglion Neurons after Selective Hair Cell Lesion. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **35**, 15050-15061, doi:10.1523/jneurosci.2325-15.2015 (2015).
83 Ikeda, M. & Morgenstern, C. Immune response of the endolymphatic sac to horseradish peroxidase: immunologic route from the middle ear to the inner ear. *The Annals of otology, rhinology, and laryngology* **98**, 975-979, doi:10.1177/000348948909801211 (1989).
84 Jung, D. H., Nadol, J. B., Jr., Folkerth, R. D. & Merola, J. F. Histopathology of the Inner Ear in a Case With Recent Onset of Cogan's Syndrome: Evidence for Vasculitis. *The Annals of otology, rhinology, and laryngology* **125**, 20-24, doi:10.1177/0003489415595426 (2016).
85 Lobo, D., Tunon, M., Villarreal, I., Brea, B. & Garcia-Berrocal, J. R. Intratympanic gadolinium magnetic resonance imaging supports the role of endolymphatic hydrops in the pathogenesis of immune-mediated inner-ear disease. *The Journal of laryngology and otology* **132**, 554-559, doi:10.1017/s0022215118000749 (2018).
86 Wackym, P. A. *et al.* Human endolymphatic sac: possible mechanisms of pressure regulation. *The Journal of laryngology and otology* **101**, 768-779, doi:10.1017/s0022215100102713 (1987).
87 Inamoto, R., Miyashita, T., Akiyama, K., Mori, T. & Mori, N. Endolymphatic sac is involved in the regulation of hydrostatic pressure of cochlear endolymph. *American journal of physiology. Regulatory, integrative and comparative physiology* **297**, R1610-1614, doi:10.1152/ajpregu.00073.2009 (2009).
88 Moller, M. N., Kirkeby, S., Vikesa, J., Nielsen, F. C. & Caye-Thomasen, P. The human endolymphatic sac expresses natriuretic peptides. *The Laryngoscope* **127**, E201-e208, doi:10.1002/lary.26074 (2017).
89 Swinburne, I. A. *et al.* Lamellar projections in the endolymphatic sac act as a relief valve to regulate inner ear pressure. *eLife* **7**, doi:10.7554/eLife.37131 (2018).
90 Maekawa, C. *et al.* Expression and translocation of aquaporin-2 in the endolymphatic sac in patients with Meniere's disease. *Journal of neuroendocrinology* **22**, 1157-1164, doi:10.1111/j.1365-2826.2010.02060.x (2010).

91 Kitahara, T., Maekawa, C., Kizawa, K., Horii, A. & Doi, K. Plasma vasopressin and V2 receptor in the endolymphatic sac in patients with delayed endolymphatic hydrops. *Otology & neurotology : official publication of the American Otological Society, American Neurotology Society [and] European Academy of Otology and Neurotology* **30**, 812-819, doi:10.1097/MAO.0b013e3181b11db5 (2009).
92 Beal, D. D., Davey, P. R. & Lindsay, J. R. Inner ear pathology of congenital deafness. *Archives of otolaryngology (Chicago, Ill. : 1960)* **85**, 134-142, doi:10.1001/archotol.1967.00760040136003 (1967).
93 Murray, L. N., Tanaka, G. J., Cameron, D. S. & Gianoli, G. J. Coronal computed tomography of the normal vestibular aqueduct in children and young adults. *Archives of otolaryngology--head & neck surgery* **126**, 1351-1357, doi:10.1001/archotol.126.11.1351 (2000).
94 Vijayasekaran, S. *et al.* When is the vestibular aqueduct enlarged? A statistical analysis of the normative distribution of vestibular aqueduct size. *AJNR. American journal of neuroradiology* **28**, 1133-1138, doi:10.3174/ajnr.A0495 (2007).
95 Boston, M. *et al.* The large vestibular aqueduct: a new definition based on audiologic and computed tomography correlation. *Otolaryngology--head and neck surgery : official journal of American Academy of Otolaryngology-Head and Neck Surgery* **136**, 972-977, doi:10.1016/j.otohns.2006.12.011 (2007).
96 Jackler, R. K. & De La Cruz, A. The large vestibular aqueduct syndrome. *The Laryngoscope* **99**, 1238-1242; discussion 1242-1233, doi:10.1288/00005537-198912000-00006 (1989).
97 Antonelli, P. J., Varela, A. E. & Mancuso, A. A. Diagnostic yield of high-resolution computed tomography for pediatric sensorineural hearing loss. *The Laryngoscope* **109**, 1642-1647, doi:10.1097/00005537-199910000-00018 (1999).
98 Levenson, M. J., Parisier, S. C., Jacobs, M. & Edelstein, D. R. The large vestibular aqueduct syndrome in children. A review of 12 cases and the description of a new clinical entity. *Archives of otolaryngology--head & neck surgery* **115**, 54-58 (1989).
99 Gonzalez-Garcia, J. A. *et al.* Enlarged vestibular aqueduct: Looking for genotypic-phenotypic correlations. *European archives of oto-rhino-laryngology : official journal of the European Federation of Oto-Rhino-Laryngological Societies (EUFOS) : affiliated with the German Society for Oto-Rhino-Laryngology - Head and Neck Surgery* **263**, 971-976, doi:10.1007/s00405-006-0095-x (2006).
100 Berrettini, S. *et al.* Large vestibular aqueduct syndrome: audiological, radiological, clinical, and genetic features. *American journal of otolaryngology* **26**, 363-371, doi:10.1016/j.amjoto.2005.02.013 (2005).
101 Lee, H. J. *et al.* Expression of anion exchangers in cultured human endolymphatic sac epithelia. *Otology & neurotology : official publication of the American Otological Society, American Neurotology Society [and] European Academy of Otology and Neurotology* **33**, 1664-1671, doi:10.1097/MAO.0b013e31826bf3d3 (2012).
102 Ramirez-Camacho, R., Ramon Garcia Berrocal, J., Arellano, B. & Trinidad, A. Familial isolated unilateral large vestibular aqueduct syndrome. *ORL; journal for oto-rhino-laryngology and its related specialties* **65**, 45-48, doi:10.1159/000068663 (2003).

103 Aschendorff, A., Marangos, N. & Laszig, R. Large vestibular aqueduct syndrome and its implication for cochlear implant surgery. *The American journal of otology* **18**, S57 (1997).
104 Lopez-Escamez, J. A. *et al.* Diagnostic criteria for Meniere's disease. *Journal of vestibular research : equilibrium & orientation* **25**, 1-7, doi:10.3233/ves-150549 (2015).
105 Havia, M., Kentala, E. & Pyykko, I. Prevalence of Meniere's disease in general population of Southern Finland. *Otolaryngology--head and neck surgery : official journal of American Academy of Otolaryngology-Head and Neck Surgery* **133**, 762-768, doi:10.1016/j.otohns.2005.06.015 (2005).
106 Yardley, L., Dibb, B. & Osborne, G. Factors associated with quality of life in Meniere's disease. *Clinical otolaryngology and allied sciences* **28**, 436-441 (2003).
107 Hallpike, C. S. & Cairns, H. Observations on the Pathology of Meniere's Syndrome: (Section of Otology). *Proceedings of the Royal Society of Medicine* **31**, 1317-1336 (1938).
108 Yamakawa, K. Ueber die patologische Veränderung bei einem Menière-Kranken. Proceedings of 42nd Annual Meeting Oto-Rhino-Laryngol Soc Japan. *J Otolaryngol Soc Jpn* **4**, 2310-2312 (1938).
109 Merchant, S. N., Rauch, S. D. & Nadol, J. B., Jr. Meniere's disease. *European archives of oto-rhino-laryngology : official journal of the European Federation of Oto-Rhino-Laryngological Societies (EUFOS) : affiliated with the German Society for Oto-Rhino-Laryngology - Head and Neck Surgery* **252**, 63-75, doi:10.1007/bf00168023 (1995).
110 Nakashima, T. *et al.* Visualization of endolymphatic hydrops in patients with Meniere's disease. *The Laryngoscope* **117**, 415-420, doi:10.1097/MLG.0b013e31802c300c (2007).
111 Naganawa, S. *et al.* Imaging endolymphatic hydrops at 3 tesla using 3D-FLAIR with intratympanic Gd-DTPA administration. *Magnetic resonance in medical sciences : MRMS : an official journal of Japan Society of Magnetic Resonance in Medicine* **7**, 85-91, doi:10.2463/mrms.7.85 (2008).
112 Gallego-Martinez, A., Espinosa-Sanchez, J. M. & Lopez-Escamez, J. A. Genetic contribution to vestibular diseases. *Journal of neurology* **265**, 29-34, doi:10.1007/s00415-018-8842-7 (2018).
113 Danckwardt-Lilliestrom, N., Friberg, U., Kinnefors, A. & Rask-Andersen, H. "Endolymphatic sacitis" in a case of active Meniere's disease. An ultrastructural histopathologic investigation. *The Annals of otology, rhinology, and laryngology* **106**, 190-198, doi:10.1177/000348949710600303 (1997).
114 Yazawa, Y. & Kitahara, M. Immunofluorescent study of the endolymphatic sac in Meniere's disease. *Acta oto-laryngologica. Supplementum* **468**, 71-76, doi:10.3109/00016488909139024 (1989).
115 Frejo, L. *et al.* Regulation of Fn14 Receptor and NF-kappaB Underlies Inflammation in Meniere's Disease. *Frontiers in immunology* **8**, 1739, doi:10.3389/fimmu.2017.01739 (2017).
116 Kim, S. H. *et al.* Autoimmunity as a candidate for the etiopathogenesis of Meniere's disease: detection of autoimmune reactions and diagnostic biomarker candidate. *PloS one* **9**, e111039, doi:10.1371/journal.pone.0111039 (2014).
117 Yamane, H. *et al.* Assessment of Meniere's disease from a radiological aspect - saccular otoconia as a cause of Meniere's disease? *Acta oto-laryngologica* **132**, 1054-1060, doi:10.3109/00016489.2012.680980 (2012).

118 Hornibrook, J. & Bird, P. A New Theory for Meniere's Disease: Detached Saccular Otoconia. *Otolaryngology--head and neck surgery : official journal of American Academy of Otolaryngology-Head and Neck Surgery* **156**, 350-352, doi:10.1177/0194599816675843 (2017).
119 Friberg, U. & Rask-Andersen, H. Vascular occlusion in the endolymphatic sac in Meniere's disease. *The Annals of otology, rhinology, and laryngology* **111**, 237-245, doi:10.1177/000348940211100308 (2002).
120 Ciccone, M. M. *et al.* Idiopathic sudden sensorineural hearing loss and meniere syndrome: The role of cerebral venous drainage. *Clinical otolaryngology : official journal of ENT-UK ; official journal of Netherlands Society for Oto-Rhino-Laryngology & Cervico-Facial Surgery* **43**, 230-239, doi:10.1111/coa.12947 (2018).
121 Wilbrand, H. F. Multidirectional tomography of the facial canal. *Acta radiologica: diagnosis* **16**, 654-672, doi:10.1177/028418517501600613 (1975).
122 Rask-Andersen, H., Stahle, J. & Wilbrand, H. Human cochlear aqueduct and its accessory canals. *The Annals of otology, rhinology & laryngology. Supplement* **86**, 1-16 (1977).
123 Atturo, F., Barbara, M. & Rask-Andersen, H. Is the human round window really round? An anatomic study with surgical implications. *Otology & neurotology : official publication of the American Otological Society, American Neurotology Society [and] European Academy of Otology and Neurotology* **35**, 1354-1360, doi:10.1097/mao.0000000000000332 (2014).
124 Atturo, F., Barbara, M. & Rask-Andersen, H. On the anatomy of the 'hook' region of the human cochlea and how it relates to cochlear implantation. *Audiology & neuro-otology* **19**, 378-385, doi:10.1159/000365585 (2014).
125 Schart-Moren, N., Larsson, S., Rask-Andersen, H. & Li, H. Anatomical Characteristics of Facial Nerve and Cochlea Interaction. *Audiology & neuro-otology* **22**, 41-49, doi:10.1159/000475876 (2017).
126 Schart-Moren, N., Larsson, S., Rask-Andersen, H. & Li, H. Three-Dimensional Analysis of the Fundus of the Human Internal Acoustic Canal. *Ear and hearing* **39**, 563-572, doi:10.1097/aud.0000000000000510 (2018).
127 Schart-Moren, N., Agrawal, S. K., Ladak, H. M., Li, H. & Rask-Andersen, H. Effects of Various Trajectories on Tissue Preservation in Cochlear Implant Surgery: A Micro-Computed Tomography and Synchrotron Radiation Phase-Contrast Imaging Study. *Ear and hearing* **40**, 393-400, doi:10.1097/aud.0000000000000624 (2019).
128 Wadin, K. & Wilbrand, H. The labyrinthine portion of the facial canal. A comparative radioanatomic investigation. *Acta radiologica (Stockholm, Sweden : 1987)* **28**, 17-23 (1987).
129 Erixon, E., Hogstorp, H., Wadin, K. & Rask-Andersen, H. Variational anatomy of the human cochlea: implications for cochlear implantation. *Otology & neurotology : official publication of the American Otological Society, American Neurotology Society [and] European Academy of Otology and Neurotology* **30**, 14-22, doi:10.1097/MAO.0b013e31818a08e8 (2009).
130 Wysokinski, T. W., Chapman, D. Adams, G. Beamlines of the biomedical imaging and therapy facility at the Canadian light source - Part 3. *Nucl. instruments Methods Fys Res Sect A Accel Spectrometers, Detect Assoc Equip* **775**, 1-4 (2015).
131 Nabeya, D. A study in the comparative anatomy of the blood-vascular system of the internal ear in Mammalia and Homo. *Acta Scholae Medicinalis, Kyoto University* **6**, 1-132 (1923).

132 Kim, H. M. & Wangemann, P. Failure of fluid absorption in the endolymphatic sac initiates cochlear enlargement that leads to deafness in mice lacking pendrin expression. *PloS one* **5**, e14041, doi:10.1371/journal.pone.0014041 (2010).
133 Wangemann, P. The role of pendrin in the development of the murine inner ear. *Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology* **28**, 527-534, doi:10.1159/000335113 (2011).
134 Rauch, S. D., Merchant, S. N. & Thedinger, B. A. Meniere's syndrome and endolymphatic hydrops. Double-blind temporal bone study. *The Annals of otology, rhinology, and laryngology* **98**, 873-883, doi:10.1177/000348948909801108 (1989).
135 Clausen, M. V., Hilbers, F. & Poulsen, H. The Structure and Function of the Na,K-ATPase Isoforms in Health and Disease. *Frontiers in physiology* **8**, 371, doi:10.3389/fphys.2017.00371 (2017).
136 Liu, W., Schrott-Fischer, A., Glueckert, R., Benav, H. & Rask-Andersen, H. The Human "Cochlear Battery" - Claudin-11 Barrier and Ion Transport Proteins in the Lateral Wall of the Cochlea. *Frontiers in molecular neuroscience* **10**, 239, doi:10.3389/fnmol.2017.00239 (2017).
137 Yamane, H. & Nakai, Y. Immunohistochemical localization of Na+-K+-ATPase in the endolymphatic sac. *Advances in oto-rhino-laryngology* **42**, 123-128, doi:10.1159/000416090 (1988).
138 Ichimiya, I., Adams, J. C. & Kimura, R. S. Immunolocalization of Na+, K(+)-ATPase, Ca(++)-ATPase, calcium-binding proteins, and carbonic anhydrase in the guinea pig inner ear. *Acta oto-laryngologica* **114**, 167-176 (1994).
139 ten Cate, W. J., Curtis, L. M. & Rarey, K. E. Na,K-ATPase subunit isoform expression in the guinea pig endolymphatic sac. *ORL; journal for oto-rhino-laryngology and its related specialties* **56**, 257-262, doi:10.1159/000276669 (1994).
140 Akiyama, K., Miyashita, T., Matsubara, A. & Mori, N. The detailed localization pattern of Na+/K+/2Cl- cotransporter type 2 and its related ion transport system in the rat endolymphatic sac. *The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society* **58**, 759-763, doi:10.1369/jhc.2010.956045 (2010).
141 Miyashita, T. *et al.* Presence of FXYD6 in the endolymphatic sac epithelia. *Neuroscience letters* **513**, 47-50, doi:10.1016/j.neulet.2012.02.005 (2012).
142 Fina, M. & Ryan, A. Expression of mRNAs encoding alpha and beta subunit isoforms of Na,K-ATPase in the vestibular labyrinth and endolymphatic sac of the rat. *Molecular and cellular neurosciences* **5**, 604-613, doi:10.1006/mcne.1994.1074 (1994).
143 Couloigner, V., Loiseau, A., Sterkers, O., Amiel, C. & Ferrary, E. Effect of locally applied drugs on the endolymphatic sac potential. *The Laryngoscope* **108**, 592-598, doi:10.1097/00005537-199804000-00024 (1998).
144 Teixeira, M. *et al.* Evidence for apical K conductance and Na-K-2Cl cotransport in the endolymphatic sac of guinea pig. *Hearing research* **128**, 45-50, doi:10.1016/s0378-5955(98)00197-x (1999).
145 Rask-Andersen, H., DeMott, J. E., Bagger-Sjoback, D. & Salt, A. N. Morphological changes of the endolymphatic sac induced by microinjection of artificial endolymph into the cochlea. *Hearing research* **138**, 81-90 (1999).
146 Rask-Andersen, H., Friberg, U., Erwall, C. & Jansson, B. Effects of hyperosmolar substances on the endolymphatic sac. *Acta oto-laryngologica. Supplementum* **468**, 49-52, doi:10.3109/00016488909139020 (1989).

147 Furuta, H. *et al.* Mineralocorticoid type I receptor in the rat cochlea: mRNA identification by polymerase chain reaction (PCR) and in situ hybridization. *Hearing research* **78**, 175-180, doi:10.1016/0378-5955(94)90023-x (1994).
148 Matsubara, A., Miyashita, T., Inamoto, R., Hoshikawa, H. & Mori, N. Cystic fibrosis transmembrane conductance regulator in the endolymphatic sac of the rat. *Auris, nasus, larynx* **41**, 409-412, doi:10.1016/j.anl.2014.02.005 (2014).
149 Akiyama, K., Miyashita, T., Mori, T., Inamoto, R. & Mori, N. Expression of thiazide-sensitive Na+-Cl- cotransporter in the rat endolymphatic sac. *Biochemical and biophysical research communications* **371**, 649-653, doi:10.1016/j.bbrc.2008.04.081 (2008).
150 Patel, M. *et al.* Intratympanic methylprednisolone versus gentamicin in patients with unilateral Meniere's disease: a randomised, double-blind, comparative effectiveness trial. *Lancet (London, England)* **388**, 2753-2762, doi:10.1016/s0140-6736(16)31461-1 (2016).
151 Kolla, V. & Litwack, G. Transcriptional regulation of the human Na/K ATPase via the human mineralocorticoid receptor. *Molecular and cellular biochemistry* **204**, 35-40 (2000).
152 Hawkins, J. E., Jr. Comparative otopathology: aging, noise, and ototoxic drugs. *Advances in oto-rhino-laryngology* **20**, 125-141, doi:10.1159/000393093 (1973).
153 Juhn, S. K., Rybak, L. P. & Prado, S. Nature of blood-labyrinth barrier in experimental conditions. *The Annals of otology, rhinology, and laryngology* **90**, 135-141, doi:10.1177/000348948109000208 (1981).
154 Harris, J. P. Immunology of the inner ear: response of the inner ear to antigen challenge. *Otolaryngology--head and neck surgery : official journal of American Academy of Otolaryngology-Head and Neck Surgery* **91**, 18-32, doi:10.1177/019459988309100105 (1983).
155 Tomiyama, S. & Harris, J. P. The endolymphatic sac: its importance in inner ear immune responses. *The Laryngoscope* **96**, 685-691 (1986).
156 Altermatt, H. J., Gebbers, J. O., Muller, C., Arnold, W. & Laissue, J. A. Human endolymphatic sac: evidence for a role in inner ear immune defence. *ORL; journal for oto-rhino-laryngology and its related specialties* **52**, 143-148, doi:10.1159/000276124 (1990).
157 Gloddek, B., Ryan, A. F. & Harris, J. P. Homing of lymphocytes to the inner ear. *Acta oto-laryngologica* **111**, 1051-1059 (1991).
158 Friis, M., Thomsen, A. R., Poulsen, S. S. & Qvortrup, K. Experimental hyperactivity of the endolymphatic sac. *Audiology & neuro-otology* **18**, 125-133, doi:10.1159/000345977 (2013).
159 Linthicum, F. in *The National Temporal Bone Registry Newsletter* Vol. vol 9 (2001).
160 Arnold, W. & Niedermeyer, H. P. Herpes simplex virus antibodies in the perilymph of patients with Meniere disease. *Archives of otolaryngology--head & neck surgery* **123**, 53-56, doi:10.1001/archotol.1997.01900010061008 (1997).
161 Selmani, Z., Marttila, T. & Pyykko, I. Incidence of virus infection as a cause of Meniere's disease or endolymphatic hydrops assessed by electrocochleography. *European archives of oto-rhino-laryngology : official journal of the European Federation of Oto-Rhino-Laryngological Societies (EUFOS) : affiliated with the German Society for Oto-Rhino-Laryngology - Head and Neck Surgery* **262**, 331-334, doi:10.1007/s00405-004-0816-y (2005).
162 Hughes, G. B., Kinney, S. E., Barna, B. P. & Calabrese, L. H. Autoimmune reactivity in Meniere's disease: a preliminary report. *The Laryngoscope* **93**, 410-417, doi:10.1002/lary.1983.93.4.410 (1983).

163 Dornhoffer, J. L. & Arenberg, I. K. Immune mechanisms in Meniere's syndrome. *Otolaryngologic clinics of North America* **30**, 1017-1026 (1997).
164 Liu, W., Kampfe Nordstrom, C., Danckwardt-Lilliestrom, N. & Rask-Andersen, H. Human Inner Ear Immune Activity: A Super-Resolution Immunohistochemistry Study. *Frontiers in neurology* **10**, 728, doi:10.3389/fneur.2019.00728 (2019).
165 Franceschi, C. *et al.* Inflamm-aging. An evolutionary perspective on immunosenescence. *Annals of the New York Academy of Sciences* **908**, 244-254, doi:10.1111/j.1749-6632.2000.tb06651.x (2000).
166 Giunta, B. *et al.* Inflammaging as a prodrome to Alzheimer's disease. *Journal of neuroinflammation* **5**, 51, doi:10.1186/1742-2094-5-51 (2008).
167 Verschuur, C. A. *et al.* Markers of inflammatory status are associated with hearing threshold in older people: findings from the Hertfordshire Ageing Study. *Age and ageing* **41**, 92-97, doi:10.1093/ageing/afr140 (2012).
168 Felder, E. & Schrott-Fischer, A. Quantitative evaluation of myelinated nerve fibres and hair cells in cochleae of humans with age-related high-tone hearing loss. *Hearing research* **91**, 19-32, doi:10.1016/0378-5955(95)00158-1 (1995).
169 Bagger-Sjoback, D. & Rask-Andersen, H. The permeability barrier of the endolymphatic sac. A hypothesis of fluid and electrolyte exchange based on freeze fracturing. *The American journal of otology* **7**, 134-140 (1986).
170 Miyamoto, H. & Morgenstern, C. Potassium level in endolymphatic sac of guinea pigs in vivo. *Archives of oto-rhino-laryngology* **222**, 77-78, doi:10.1007/bf00456341 (1979).
171 Rask-Andersen, H. The function of the endolymphatic duct. *In proceeding of the 1st International Meeting on Meniere´s Disease held in Dusseldorf.* (1980).
172 Kimura, R. S., Schuknecht, H. F., Ota, C. Y. & Jones, D. D. Obliteration of the ductus reuniens. *Acta oto-laryngologica* **89**, 295-309, doi:10.3109/00016488009127141 (1980).
173 Knapp, H. J. A clinical analysis of the inflammatory affections of the inner ear. *Archives of Ophtalmology and Otology* **2**, 204-283 (1871).
174 Rask-Andersen, H., Friberg, U., Johansson, M. & Stjernschantz, J. Effects of intratympanic injection of latanoprost in Meniere's disease: a randomized, placebo-controlled, double-blind, pilot study. *Otolaryngology--head and neck surgery : official journal of American Academy of Otolaryngology-Head and Neck Surgery* **133**, 441-443, doi:10.1016/j.otohns.2005.05.043 (2005).
175 Merchant, S. N., Nadol, J.B. *Schuknecht's pathology of the ear.*, (People's medical publishing house., 2010).
176 Hosseinzadeh, M., Hilinski, J. M., Turner, W. J. & Harris, J. P. Meniere disease caused by an anomalous vein of the vestibular aqueduct. *Archives of otolaryngology--head & neck surgery* **124**, 695-698, doi:10.1001/archotol.124.6.695 (1998).
177 Ostrowski, V. B. & Kartush, J. M. Endolymphatic sac-vein decompression for intractable Meniere's disease: long term treatment results. *Otolaryngology--head and neck surgery : official journal of American Academy of Otolaryngology-Head and Neck Surgery* **128**, 550-559, doi:10.1016/s0194-59980300084-6 (2003).

Acta Universitatis Upsaliensis

Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine 1647

Editor: The Dean of the Faculty of Medicine

A doctoral dissertation from the Faculty of Medicine, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine. (Prior to January, 2005, the series was published under the title "Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine".)

Distribution: publications.uu.se
urn:nbn:se:uu:diva-406848

ACTA
UNIVERSITATIS
UPSALIENSIS
UPPSALA
2020