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COCHLEAR IMPLANTATION: EXPERIMENTAL AND CLINICAL STUDIES

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Cochlear Implantation: Experimental and Clinical Studies

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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To Birger, 1913-1998,

for teaching true values of life

ABSTRACT

Cochlear implantation makes hearing restoration possible in patients with severe to profound hearing loss. However, patients with residual hearing, where a cochlear implant may be combined with acoustic stimulation, and children with malformed cochleae, where the surgery itself as well as language training may be a challenge, are two important groups of patients that require special procedures. These patient groups are the subject of this thesis.

The first study (paper I) examined the effects of cochlear implantation on residual hearing and postoperative histology in a guinea pig model. After mild to moderate levels of surgical trauma, effectuated as a cochleostomy alone or in combination with limited electrode array insertion, hearing recovered after a two-week period of loss (a temporary threshold shift). The intracochlear structures remained unchanged. A second study (paper II) was performed to test the hypothesis that cochlear implantation may induce endolymphatic hydrops, which could lead to hearing loss. The results indicate that hydrops is present during the first week after cochlear implantation.

These experimental studies conclude that the guinea pig cochlea shows high resilience to cochlear implantation and that mechanical damage incurred during surgery does not explain the loss of residual hearing often seen in patients. Secondary mechanisms, such as hydrops, are likely to be involved in the early postoperative period. This information is important as patients with useful residual hearing increasingly receive cochlear implants.

Two clinical studies examined the effects of cochlear implant surgery on children with x-linked inner ear malformation. The first of these (paper III) describes surgical techniques necessary for safe cochlear implantation, and further shows that implantation permits hearing restoration and the development of spoken language in these children. Further analysis of hearing and language outcomes, cognition and mental health (paper IV) revealed poorer outcome in hearing, language and mental health and lower executive functional level, as compared to a control group. Genetic analysis confirmed mutations in the POU3F4 gene on the X-chromosome.

X-linked malformation deafness is usually considered non-syndromic. However, paper IV shows that these children exhibit signs of neuro-developmental problems consistent with attention deficit and hyperactivity, which is likely related to the POU3F4 mutation. Hence, x-linked cochlear malformation should be re-classified as a syndromic form of hearing loss.

LIST OF PUBLICATIONS

I. Smeds H, Fransson A, Ulfendahl M, Fridberger A. Differences in the extent of injury during cochlear implantation alters hearing in a guinea pig model. *Manuscript*

II. Smeds H, Eastwood H T, Hampson A J, Sale P, Campbell L J, Arhatari B D, Mansour S, O'Leary S J. Endolymphatic hydrops is prevalent in the first weeks following cochlear implantation. *Hearing Research 327 (2015) 48-57*

III. Smeds H, Wales J, Asp F, Löfkvist U, Falahat B, Anderlid B-M, Anmyr L, Karltorp E. X-linked Malformation and Cochlear Implantation. *Otology & Neurotology, ePub ahead of print*

IV. Smeds H, Karltorp E, Anderlid, B-M, Henricson C, Asp F, Anmyr L, Wales J, Lagerstedt-Robinson K, Malmgren H, Löfkvist U. X-linked malformation deafness, a new syndrome? *Manuscript*

LIST OF CONTENTS

ABBREVIATIONS

ABI	Auditory Brainstem Implant
ABR	Auditory Brainstem Response
BRIEF	Behavior Rating Inventory of Executive Function
CAP	Compound Action Potential
CI	Cochlear Implant
CH	Cochlear Hypoplasia
CSF	Cerebrospinal Fluid
CT	Computed Tomography
dB	Decibel
dBA	Decibel Attenuation
DFNX	DeaFNess on the X-chromosome
DNA	Deoxyribonucleic Acid
ECochG	Electrocochleography
FM	Frequency Modulation
IHC	Inner Hair Cell
IAC	Internal Auditory Canal
IP	Incomplete Partition
LVA	Large Vestibular Aqueduct
MAIS	Meaningful Auditory Integration Scale
Micro-CT	Micro- Computerized Tomography
MRI	Magnetic Resonance Imaging
HL	Hearing level
OHC	Outer Hair Cell
PTA	Pure Tone Average
PTAlow	Pure Tone Average of low frequencies (125, 250 and 500 Hz)
RHS	Radiological Hydrops Score
RNA	Ribonucleic Acid

RW	Round Window
SCC	Semicircular canals
SDQ	Strength and Difficulties Questionnaire
SGN	Spiral Ganglion Neuron
SM	Scala Media
SMAD	Scala Media Area Difference
SP	Summation Potential
SPL	Sound pressure level
SV	Scala Vestibuli
ST	Scala Tympani
TEA-Ch	Test of Everyday Attention for Children

INTRODUCTION

The cochlear implant replaces a non-functioning inner ear. This invention has revolutionized the possibility of hearing rehabilitation for patients with severe to profound deafness. During the last decades hundreds of thousands of patients have had the opportunity to hear through electrical stimulation of the auditory nerve mediated by the implant. Children born deaf are today, to a very large extent, able to learn to hear, speak and attend mainstream schools, and children or adults who have turned deaf have the possibility to return to a life with functional hearing. The cochlear implant has without doubt changed our view on deafness and opened a new field of treatment.

I remember my first encounter with a patient at first switch-on of a sound processor a couple of weeks after cochlear implant surgery. After having been deaf for twenty years she could, from one moment to the other, hear when someone spoke to her, and even talk to her relatives on the phone! I was truly amazed, and realized that this was something I absolutely had to learn more about.

In spite of the good results for many patients, there are challenges remaining. This thesis describes two particular patient groups with special difficulties relating to their cochlear implant treatment. The first of these two relate to understanding the mechanisms of hearing loss after surgery to the inner ear. Patients with useful residual hearing may, if the hearing is preserved at surgery, combine electrical stimulation with acoustic “normal” hearing with benefit. However, the patient may lose this possibility as the surgery sometimes induce hearing loss, rapid or slowly progressive. The two first papers report on this topic. They are experimental with the aim of understanding possible actions relating to loss of hearing during cochlear implant surgery.

The second group with specific challenges are children born deaf because of inner ear malformations. The difficulties displayed in this subgroup of pediatric cochlear implant recipients include both surgical and post-operative training aspects. This thesis address children with x-linked malformation of the inner ear. The last two papers are clinical, describing the surgical method and outcome of cochlear implantation, aiming to give a comprehensive picture for this group of children.

BACKGROUND

THE EAR AND NORMAL HEARING

In humans, the ear is the organ to register pressure fluctuations within a frequency range we call “sound”. The perception of sound requires a functioning hearing organ and auditory neural pathways. In addition, to make the sound meaningful, the brain has to interpret the nerve signal for the individual to understand what it means. Thus, we are born with the anatomy to listen, but we learn to hear.

Sound, to humans, are vibrations within the frequency range of 20-16000 Hz transmitted through a medium, usually air in our everyday life. In an ear with normal function the vibrations, propagated as sound pressure alterations, “waves”, reaches the pinna and external auditory canal, resulting in movement of the tympanic membrane. These movements are transmitted to the inner ear by the ossicular chain located in the air-filled middle ear. The fluid-filled inner ear contains the sensory epithelium for both hearing and balance. Because the fluid is non-compliant the vibrations are routed towards the hearing organ, the cochlea, as the mechanical impulses there may be equalized by round window movements. As there is normally no such alternative opening in the balance system all sound energy may be picked up by the hearing organ (Figure 1).

The inner ear, capsula otica, is firmly embedded in the very hard petrosal part of the temporal bone. The spiral shaped bony labyrinth is filled with perilymph with the membranous labyrinth, winding inside, containing endolymph. Inside of the cochlea the fluid compartments are divided by the basilar membrane and Reissner’s membrane. In cross section, this gives three separate canals, “scalae”, with the hearing organ, the organ of Corti, being located on the basilar membrane with its sensory epithelium, the hair cells, facing the endolymphatic space, the scala media. With specific mechanical properties, changing along its length, the anatomy of the basilar membrane constitutes the base for the separation of frequencies, the tonotopy, of the cochlea. A specific frequency of sound will produce a travelling wave along the membrane and the physical properties at a certain point of the membrane will be susceptible for those frequency vibrations creating a wave maximum. The basilar membrane is stiffer in the basal turn corresponding to higher frequencies, and broader, thinner, and less stiff towards the apex, creating a wave maximum for lower frequencies. The hair cells in the region of a vibrating basilar membrane will react as the stereocilia, attached to the tectorial membrane, will bend and activate depolarization (Fridberger *et al.*, 2006), initiating an action potential in the dendrite of the spiral ganglion neuron (SGN) propagating as a nerve signal in the axon. Multiple axons constitute the auditory nerve running in the internal auditory canal, entering the posterior fossa of the skull base and attaching to the brain stem, there connecting to the cochlear nucleus (Ulfendahl, 1997, Robles *et al.*, 2001).

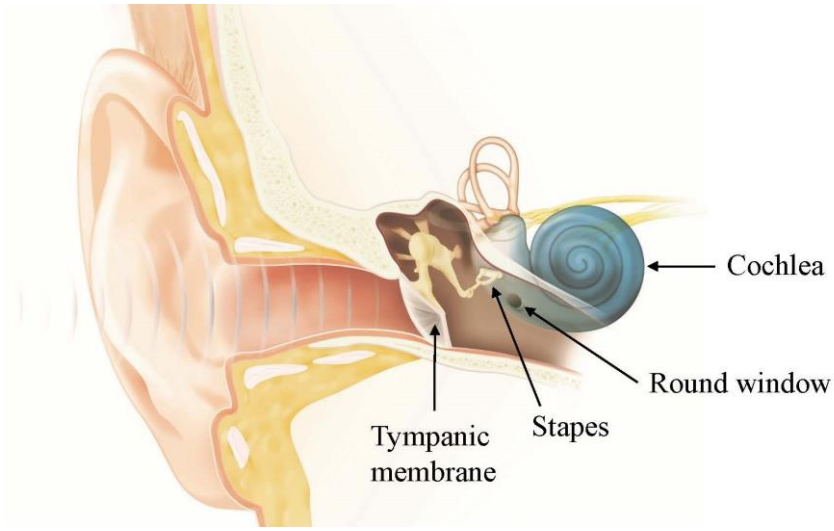

Figure 1) Cross section of the anatomy of the human ear. The cochlea is colored blue. The third bone in the ossicular chain, the stapes, is seen attaching to the inner ear by the oval window. Adjacent to that, the round window is seen as a dark hole in the basal turn of the cochlea. Figure printed with permission from Cochlear Ltd © 2016

The hair cells of the cochlea are arranged in three rows of outer hair cells (OHC) and a single row of inner hair cells (IHC). The human ear can detect a sound pressure of 20 µPa in its most sensible area, around 2000-4000 Hz (Gelfand, 2004). This area corresponds well to most of the sounds in human speech. (Obviously, as our sense of hearing and our speaking organ, the vocals cords and the upper respiratory tract, have evolved together.) The lowest detectible true intensity of sound, measured in decibel (dB) Sound pressure level (SPL), varies widely over the frequency range.

Most mammals have the same principal organization of the inner ear, however with large variations in sensitivity to frequency range and sound pressure. In this thesis the inner ear of the guinea pig has served as a model for experimental studies. The guinea pig is a common animal model in auditory research, because much of its hearing range overlaps with the human range. Furthermore, the cochlea is relatively accessible in the guinea pig, which facilitates experiments.

HEARING LOSS

Hearing loss is a very common disorder, in Sweden and worldwide. In Sweden it is today approximated that 13% of the population has a hearing loss and half of them to an extent where they need hearing aids. Approximately 0.15% of the population is completely deaf. In all, this makes hearing loss one of the most common disabilities among the population of Sweden.

Inability to hear may be related to three different principle problems or a combination of these. *Conductive* hearing loss means there is something wrong with the mechanical portion of the hearing, the pathway of the sound from the outer ear through the middle ear. Sensory hearing loss relates to malfunction in the cochlea and neural hearing loss to problems in the auditory nerves and central auditory pathways. The last two reasons are often described as *sensorineural*. A hearing loss may also be *mixed* with a combination of conductive and

sensorineural components. In addition, the term central hearing loss may be used for processes of poor cerebral processing of the sound. The hearing loss may be single-sided or bilateral.

The average normal sensitivity to sound has been set to a nominal 0dB Hearing level (HL) and a hearing loss as additional intensity necessary above that level to detect a sound. 0-20 dB HL is regarded a normal hearing. The grading of hearing loss is mild (20-40 dB HL), moderate (40-65 dB HL), severe (65-90 dB HL) and profound (>90 dB HL). When describing a hearing loss it may be called, for example, "moderate-severe" as it may be moderate in the lower frequencies and severe in the higher. To average the loss, a pure tone average (PTA) of the frequencies 500, 1000, 2000 and 4000 is often calculated. Cochlear implant candidates in general have a bilateral severe-profound sensorineural hearing loss with a PTA over 80dB as a majority of the hair cells of the cochlea are absent or non-functioning. Acoustic amplification is thereby not an option. However, the spiral ganglion neurons located in the modiolus (in a cochlea with normal anatomy) are present and susceptible to electrical stimulation.

This thesis discusses one specific *type* of hearing loss, partial deafness, and one specific *reason* for hearing loss, a genetic inner ear malformation called x-linked deafness.

Partial deafness

With age all humans gradually lose some of the ability to hear the very highest frequencies, 10-16 kHz. Such high frequency loss has little effect on everyday life communication as the frequencies of the sounds in normal speech and everyday life are located in the region of 125-8000 Hz.

The term "partial deafness" relates to a type of hearing loss where the cochlea has little or no function in one part and a relatively good function in another. Usually these patients present with severe-profound hearing loss in the frequencies above 500-1000 Hz and close to normal hearing at frequencies lower than that (Figure 2). Speech perception is very limited, as these patients are unable to detect much of the higher speech frequencies, the area of the consonants responsible for much of the discrimination of words. Amplification with conventional hearing aids offers very limited help as the non-functioning areas of the cochlea have little or no remaining hair cells. The patients are regarded difficult to rehabilitate as acoustic stimulations is not an option in the frequency areas where amplification is necessary. The majority of partial deafness patients are adults where the partial hearing loss has occurred after childhood, and they thereby have normal speech. Some cases are congenital or with early onset and these children develop an affected speech, related to limited consonant recognition. To evaluate the level of hearing loss in patients with partial deafness a PTA of the lower frequencies in the audiogram, 125, 250 and 500 Hz, is used for average (PTAlow).

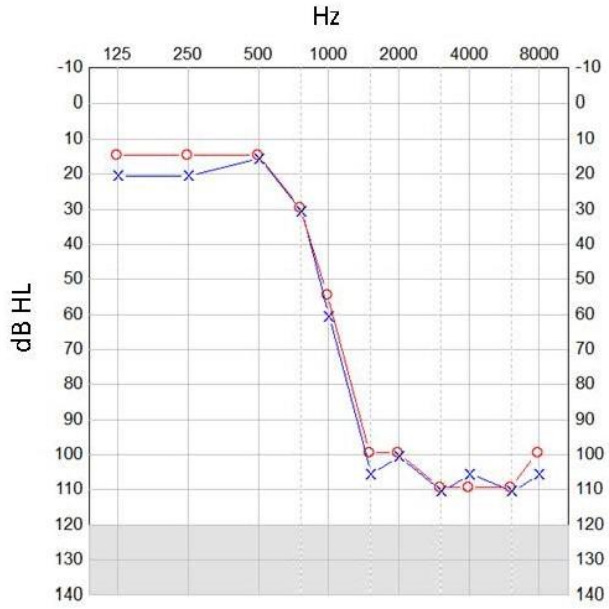

Figure 2) Pure tone audiogram of a patient with partial deafness. The hearing thresholds are normal in the 125-500 Hz region and very high in the middle- and high frequency region. Red circle- right ear, Blue cross- left ear. (PTAlow; right ear 15 dB, left ear 18 dB)

Genetic hearing loss

Approximately 2/1000 children are born with severe-profound hearing loss and in the developed world more than 50% of prelingual deafness has a genetic base. The genetic forms of hearing loss may, in addition to otologic examination and audiometry, be suspected by a family history with many affected members. A pedigree may reveal a hereditary pattern and the traits are traditionally divided into autosomal dominant or recessive, x-linked or mitochondrial. The hearing loss may be an isolated symptom or part of a syndrome with additional defects. The most common forms of genetic hearing loss are autosomal recessive and non-syndromic. Today molecular testing for genetic disease is increasingly common and a large number of mutations in genes responsible for hearing loss are known.

Hereditary hearing loss is most often sensorineural as the genetic anomaly in general affects the function of the sensory epithelium, the hair cells, but may be mixed or conductive if a simultaneous or isolated malformation of the middle or outer ear is present. Most genetic hearing loss is present at birth (prelingual) but may be progressive or with late onset once the child has learned to talk (postlingual). Although non-syndromic hearing impairment is more common than syndromic, more than 400 genetic syndromes that include hearing loss have been described (Toriello *et al.*, 2004).

X-linked deafness is rare (1-5%), compared to the vast majority of autosomal deafness. It may be syndromic, as in Alport or Mohr-Tranebjerg syndrome, but the majority of X-linked hearing loss is non-syndromic. Examples of the latter are DFNX1-DFNX4 (Petersen *et al.*, 2008), and the most common of these (50%) is DFNX2 that gives a congenital, progressive mixed severe-profound hearing loss (Figure 3).

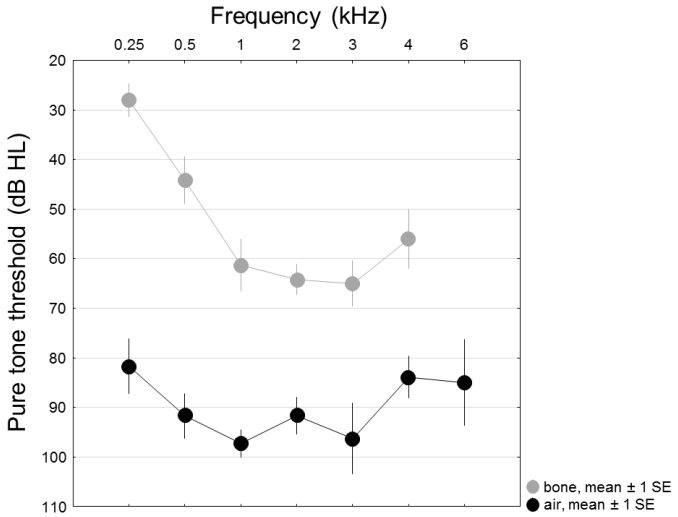

Figure 3) Preoperative air- and bone behavioral hearing thresholds as a function of frequency (visual reinforcement audiometry) in children with x-linked deafness (paper III and IV). Mean and SEM for each frequency.

On imaging of the temporal bone a typical malformation of the inner ear is seen (Gong *et al.*, 2014) classified as Incomplete Partition, type 3 (IP3) (Sennaroglu *et al.*, 2006). DFNX2 is the only form of x-linked hearing loss to give a bony malformation of the inner ear. In DFNX2 mutations in the gene *POU3F4* were identified already in 1995 by de Kok and co-workers (de Kok *et al.*, 1995). Naturally, most children with symptoms from a mutation in a gene on the X-chromosome are boys, and accordingly the majority of patients with DFNX2 are male. The role of *POU3F4* (POU domain, class 3, transcription factor 4 (Brain-4)) is to encode a transcription factor which has a role in cellular regulation by binding to DNA, controlling the coding of genetic information from DNA to RNA. *POU3F4* is part of a larger group of genes, the POU domain genes, controlling early development, however, the specific regulatory role of *POU3F4* is not fully clarified. It is described to have a function during very early stages of embryogenesis, linked to the development of the neural tube (neuroepithelial cells) (Choi *et al.*, 2013) and is found in the forebrain, including the supraoptic and paraventricular nuclei of the hypothalamus in rat studies (Mathis *et al.*, 1992). It is also found to be expressed in the periotic bone (later capsula otica) and the fibrocytes of the cochlear duct (Phippard *et al.*, 1998). It is thought to have a role in cell signaling during labyrinthine development necessary for spiral ganglion innervation. Animal studies on *pou3f4* gene knock-out mice have revealed bony inner ear (Phippard *et al.*, 1998) and spiral ligament (Minowa *et al.*, 1999) deformities. The later may affect potassium ion homeostasis. In humans, an increasing number of *POU3F4* mutations are reported. These include intragenic missense or frameshift mutations but also deletions in the gene or in the upstream regulatory element.

COCHLEAR MALFORMATIONS

The normal shaped cochlea has a well-defined bony modiolus harboring the spiral ganglion neurons and the afferent axons of the beginning of the auditory nerve. The nerve passes a bony cribriform plate at the fundus separating the cochlea from the internal auditory canal, and thereby acts as a border for the intracranial cerebrospinal fluid (CSF) and the perilymphatic

space inside of the cochlea. The internal structure is well-organized in three separate canals, “scala”, as described above, scala vestibuli, scala media and scala tympani.

Deviations from the normal anatomy are referred to as malformations. The inner ear develops in gestation week three to eight and disturbances during this period will result in an abnormal anatomy. In some cases, there is a clear genetic cause to the malformation but in others it is unclear why the inner ear is malformed and the hearing loss may be the only symptom. Hearing loss related to a malformation is always congenital but may in some cases be mild at birth and progress during life.

Diagnosis and classification depends on reliable imaging, high resolution CT and MRI. Limitations to computed tomography include soft tissues as they are poorly visualized as opacities only. The CT examination gives answer to size, the intracochlear lumen, the modiolus and the bony parts of the partitions inside of the lumen. 3 Tesla MRI adds substantial soft tissue information but resolution is still limited regarding intracochlear fibrous and neural structures. Nonetheless, the results of imaging today allow a more differentiated classification.

Classifications

In 1791 Carl Mondini first described a deformity of the inner ear (Mondini, 1791). Cochlear malformations are sometimes still inappropriately described as a “Mondini malformation” regardless type of abnormality. In 1987 Jackler and co-workers suggested a classification (Jackler *et al.*, 1987) introducing divisions of the cochlear malformations common cavity, incomplete partition and hypoplasia. In addition, there are two rare, severe deformities of the temporal bone, Michel deformity and cochlear aplasia where, in both cases, the cochlea is absent. Sennaroğlu and Saatci proposed a more precise classification in 2002 (Sennaroglu *et al.*, 2002),where the incomplete partition group is further subclassified.

In the Sennaroğlu classification a cochlea with incomplete partition (IP) has a relatively normal size of its external borders and there are three subgroups, type 1 with a cystic cochlea and a severe modiolar dysplasia, type 2 with a defined basal turn but a bud-shaped apex and a moderate modiolar dysplasia and type 3 with a cork screw appearance of the cochlea, modiolar aplasia, absent cribriform bone in the fundus and a wide internal auditory canal. In addition hypoplastic cochleae are grouped in type 1-3, with type 1 being the most severe. The Sennaroğlu classification is today widely accepted and will be used in this thesis.

IP 1 is often referred to as a cystic cochlea-vestibular malformation, type 2 is the classic Mondini malformation and coexists with a large vestibular aqueduct and type 3 is related to a mutation in the *POU3F4* gene on the X-chromosome. Paper III and IV investigates a group of children with *POU3F4* related deafness and subsequently, in this thesis, incomplete partition type 3 is discussed more in detail.

As mentioned above children with x-linked malformation are born with a mixed hearing loss, often rapidly progressing to a severe-profound level. As their hearing loss is identified on neonatal screening and auditory brainstem response (ABR) they may be deemed as suitable for a cochlear implant and referred for imaging of the temporal bone. As the imaging, starting with an MRI and, in cases of anomaly, followed by a CT, reveals a malformation the diagnosis is clear but may be additionally confirmed with genetic testing.

On imaging of the temporal bone a typical malformation of the inner ear is seen (Figure 4) (Gong *et al.*, 2014), classified as Incomplete Partition, type 3 (IP3) (Sennaroglu *et al.*, 2006).

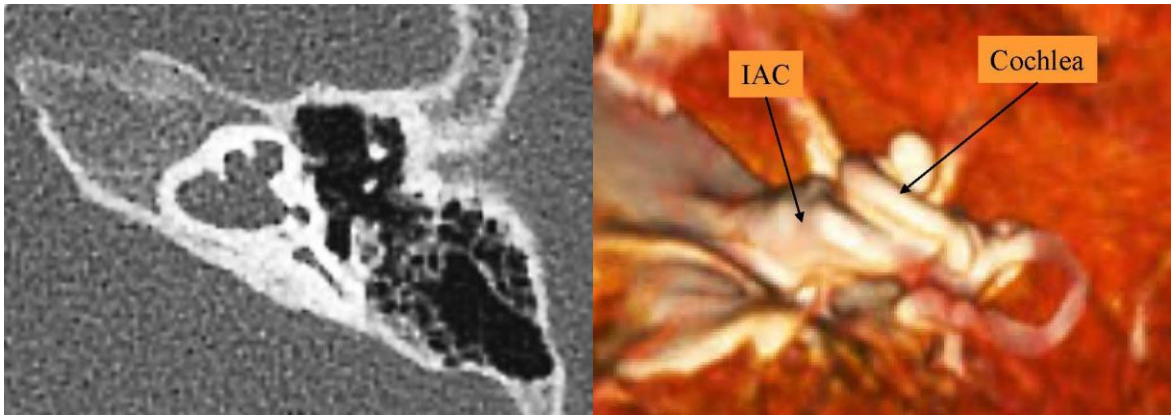

Figure 4) X-linked malformation of the inner ear. Axial CT image demonstrating a cystic malformation with modiolar aplasia (left image) and 3D-reconstruction of an MRI (right image) with the perilymphatic space colored white demonstrating an abnormal cork screw appearance of the cochlea and a very wide internal auditory canal (IAC). Courtesy of Babak Falahat, DDS, Karolinska University Hospital

In recent years all pediatric cochlear implant recipients with a cochlear malformation has been assessed, implanted and followed at Karolinska University Hospital in Stockholm. This has opened a unique opportunity for a comprehensive overall approach to this subgroup of patients.

COCHLEAR IMPLANTS

A cochlear implant is an electric device which turns sound into electrical impulses. It consists of an external part, the sound processor, and internal implant, the receiver-stimulator and the electrode array. In principle the sound is picked up by microphones on the sound processor and is after processing sent through the skin as a frequency modulation (FM)-signal to the implant by an antenna behind the ear (Figure 5). The coil of the implant picks up the signal and converts it into electrical impulses sent out in an array positioned in the cochlea (Clark, 2006). The sound frequencies are divided into channels, 12, 16 or 22 depending on the manufacturer, and each channel is lead to a specific electrode on the array, thus separating the intracochlear electrical stimulation according to tonotopy. The electrodes stimulate the neurons of the spiral ganglion in the modiolus with electrical currents according to the frequencies in the envelope of the sound (Wilson *et al.*, 1991).

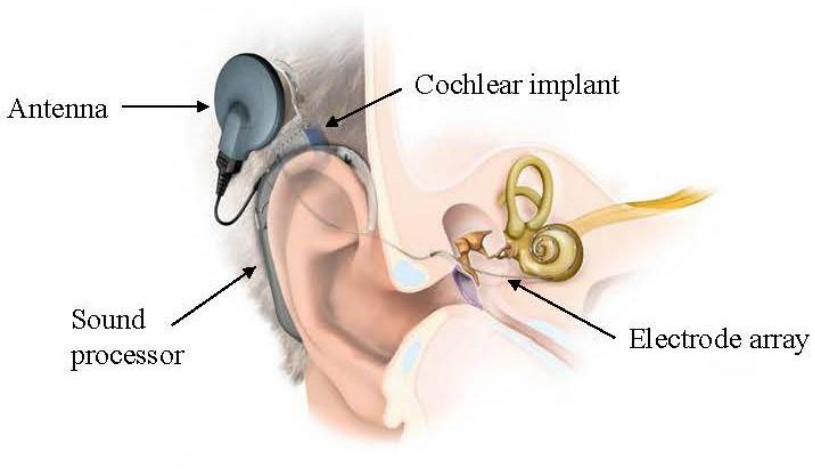

Figure 5) Cross section of a cochlear implant. The sound processor and external antenna is seen behind the ear. A transparent image of the receiver-stimulator is noticed under the hair and the electrode array is out lined trough the bone and middle ear and entering the cochlea. Figure printed with permission from MED-EL © 2016

There are several electrode array options of for implantation. Electrodes with a prefixed curvature give a perimodiolar positioning inside the cochlea whereas straight electrodes remain along the lateral wall of the scala tympani. The electrodes also have alternative lengths, 20-31 mm, depending on if a deep insertion is attempted or not. Short electrodes, around 20 mm, have been used in cases of residual hearing in the low frequencies aiming at hearing preservation.

Cochlear implants may be used uni- or bilaterally. In unilateral cases it may be combined with a conventional hearing aid on the other ear if this is experienced as a benefit by the patient.

COCHLEAR IMPLANTATION SURGERY

Cochlear implantation in patients is a standardized procedure in general anesthesia. A postauricular incision and transmastoid approach including a posterior tympanotomy is the most common access to the middle ear and the basal turn of the cochlea, the promontory. The opening to the scala tympani can be performed by drilling a hole in the promontory, a cochleostomy, or by incising the round window membrane. The electrode array of the cochlear implant may thereby be inserted into the cochlea following the anatomical boundaries of the scala tympani. Soft tissues are used to pack for sealing around the electrode. The receiver-stimulator package is positioned on the cranium in a subperiosteal pocket followed by wound closure in multiple layers. Intraoperative electrophysiological testing confirms the implant integrity and neural response of the auditory pathway. Postoperative x-ray verifies a correct positioning of the electrode inside the cochlea. The patient is given one dose of intravenous antibiotics.

Surgery and residual hearing

In the case of a patient with residual hearing, as for patients with partial deafness as described above, the aim during surgery is to preserve the remaining auditory function. This allows the use of acoustic amplification and electrical stimulation in the same ear. The concept of “soft surgery” to the cochlea was developed by Lehnhardt (Lehnhardt, 1993) and in 1999 von Ilberg

described the possibility of combined electrical and acoustic, bimodal, stimulation (EAS®) in the same ear (von Ilberg C, 1999). This type of hybrid hearing requires a postoperative result of better than 80 dB HL in the frequencies of amplification, often 125, 250 and 500 Hz, for a useful residual hearing. The concept today includes meticulous care not to touch the ossicular chain, careful exposure of the round window membrane or endosteum of the scala tympani, the use of local application of cortisone in the middle ear, incision of the round window membrane or endosteum and slow insertion of the electrode array. Postoperative steroids are administered for one week.

It is well described that patients with partial deafness benefit from a CI alone compared to preoperative use of a hearing aid (Cullen *et al.*, 2004, Dowell *et al.*, 2004). This is important as not all patients preserve their residual hearing. In a review of cochlear implantation in patients with residual hearing Talbot and Hartley concluded that 13% of the recipients sustain a total loss of residual hearing and 24% > 20dB. (Talbot *et al.*, 2008). In recent years, clinical studies evaluated long-term outcomes of cochlear implantation in patients with residual hearing. Mertens *et al.* (Mertens *et al.*, 2014) described a group of 9 patients (11 ears), followed for 10 years, showing complete low-frequency hearing preservation in 27%, partial preservation in 45%, minimal in 18% and loss of residual hearing in one subject. This study used the HEARRING group Hearing Preservation Classification System (0% = loss of hearing; >0%-25% = minimal HP; >25%-75% = partial HP; >75% = complete HP) (Skarzynski *et al.*, 2013) to evaluate hearing preservation rates and compare results of different strategies of intervention. Helbig and co-workers analyzed 103 ears (96 patients) up to eleven years after implantation (Helbig *et al.*, 2016). 12 month results (n=81) showed 31% complete hearing preservation, 48% partial and 13% minimal. Eight percent exhibited total loss of residual hearing. In a ten-year follow-up (n=62) 27% had complete preservation, 39% partial and 15% minimal. In patients with total hearing loss, no association was seen to etiology or surgical approach. In this study PTAlow shifts ≤10dB are regarded as complete preservation, between 10 and 30dB as partial and ≥30dB as minimal. Regarding auditory outcome, several authors showed that with preserved hearing, a combined stimulation is of benefit to the auditory outcome (Lorens *et al.*, 2008, Gstoettner *et al.*, 2009, Gifford *et al.*, 2013). These postoperative studies examine hearing with the CI alone, HA alone and in combination with bimodal stimulation in one ear.

The loss of residual hearing in the early or late postoperative period continues to be a problem in spite refinement of surgical technique (Skarzynski *et al.*, 2007), electrode designs (Wanna *et al.*, 2014) and use of intraoperative drugs (Ye *et al.*, 2007, James *et al.*, 2008, Connolly *et al.*, 2011). The underlying intracochlear mechanisms remain unclear. The surgical trauma, including the insertion of the electrode, may induce direct mechanical damage and activate inflammatory or cell death pathways (Eshraghi *et al.*, 2006). The presence of an electrode in scala tympani also cause fibrosis (O'Leary *et al.*, 2013). This, in combination with the electrode volume itself, is likely to generate an “intracochlear” conductive hearing loss. Paper I and II in this thesis investigates patterns of loss of residual hearing after cochlear implantation (I) and a possible mechanism responsible for alterations in hearing (II).

Surgery in malformed cochleae

Due to the variant anatomy of the malformed cochlea, a cochlear implantation may constitute a challenge. There are several aspects to consider. These cases are almost always pediatric with

the special needs related to that, especially as these children may have additional handicaps. In rare cases cranio-facial or upper airway malformations are present and anesthesia has to be planned accordingly. Abnormalities, other than the inner ear malformation, may co-exist in the temporal bone, the most important being a deviant facial nerve route or aberrant veins, making the approach challenging. The cochlea itself may be opened by the RW or a cochleostomy depending on visualization. The choice of electrode depends on the size of the cochlea, the type of malformation, and whether cerebrospinal fluid (CSF) gusher is present. The latter necessitates meticulous sealing around the electrode. All severe malformations require intraoperative x-ray to confirm the electrode position.

SPEECH, LANGUAGE, COGNITION AND MENTAL HEALTH

The ability to hear is necessary for speech development. Without early stimulation of the auditory system, the brain loses the ability to analyze and interpret signals from the auditory nerve. During the first years of pediatric cochlear implantation, the age of implantation was often four or five years and, although the children did learn to hear, their speech and language development was limited (Nicholas *et al.*, 2007). With adoption of earlier implantation age, the hearing results have become increasingly better and results today often include excellent open set speech recognition. Today, as a general rule, the spoken language of children with a cochlear implant is often at the level of age-equivalent peers in terms of intelligibility, vocabulary and fluency and many attend mainstream schools (Dettman *et al.*, 2016). However, it is important to remember that these children still suffer from hearing impairment making listening difficult, especially in noisy situations. Also, there are subgroups with special needs or handicaps requiring additional support and special schools. Furthermore, some children are still operated late due to immigration, adoption or similar reasons and multiple languages used in a family also make spoken language development more difficult (Teschendorf *et al.*, 2011).

The clinical experience of meeting children with x-linked malformation is that this group exhibit special features, including hyperactivity and attention problems. Children with hearing loss may display a similar picture. However, the x-linked group, seen in clinic at follow-up, resemble each other with a behavior different from most other pediatric CI recipients.

AIMS

This thesis considers two different aspects of cochlear implantation. The first two papers, I and II, are experimental and address the problem of hearing preservation in patients with residual hearing who receive a cochlear implant. To investigate this, a guinea pig model for cochlear implantation was used. The second aspect of the thesis relates to cochlear implantation in malformed cochleae. Paper III and IV are clinical studies investigating a group of children with severe-profound hearing loss related to x-linked malformation. These studies explore their treatment with cochlear implantation and if they require special attention during surgery and follow-up.

Paper I. The aim was to study the effects of different levels of trauma during cochlear implantation on residual hearing and cochlear histology.

Paper II. The hypothesis of this study was that endolymphatic hydrops, induced by cochlear implantation, may be responsible for the delayed hearing loss and vertigo seen in some CI recipients with residual hearing. The aim was to determine if signs of hydrops were present in a guinea pig model after cochlear implantation.

Paper III. X-linked malformation is an unusual reason for severe-profound hearing loss in children. To date only a few cases have been described in the literature. Treatment with cochlear implants has been described as related to risks of complications during surgery and the reported outcome doubtful. The purpose of this study was to describe a surgical method, evaluate complications and determine hearing outcomes with cochlear implants.

Paper IV. This paper in depth investigates the language outcome, cognitive abilities and mental health in a group of children with x-linked malformation compared to a control group. Their *POU3F4* gene mutation is analyzed with the hypothesis of this being a syndromic hearing loss.

METHODS

SURGICAL PROCEDURES

Guinea Pigs (Papers I and II)

In the experimental set-up for papers I and II adult Duncan-Hartley guinea pigs were used, albino in paper I and tricolor in paper II. The animals were housed at the animal departments of Karolinska University Hospital (paper I) and the Royal Victorian Eye and Ear Hospital (paper II) respectively. The experiments were performed in accordance with ethical standards of the Animal Care Committee in Stockholm, Sweden (paper I, Ethics approval: N12/10) and the Animal Research Ethics Committee, Melbourne, Australia (Paper II, Ethics approval: 12/261AR). The animals were anaesthetized with ketamine 40mg/kg and xylazine 10mg/kg (paper I) or ketamine 60 mg/kg and xylazine 4 mg/kg (paper II) given as intramuscular injections. The same anesthesia was used during surgeries as during later hearing testing.

In cochlear implantation in a guinea pig, the bulla is exposed with a postauricular incision. The bone of the bulla was removed with a knife or a burr (bullostomy). A cochleostomy was drilled on the promontory to open the scala tympani and an electrode array inserted. Soft tissue was applied around the electrode to seal the opening. The wound was closed with resorbable sutures and the animals received an intraperitoneal injection with 0.2 ml buprenorphine 0.3 mg/ml, a subdermal injection of 5 ml Saline 0.9% subcutaneously and 1 ml doxycykline (2 mg/ml).

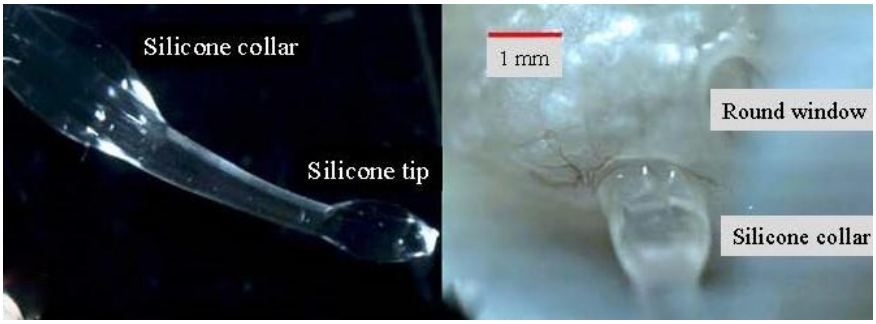

Figure 6) Guinea pig cochlear implant electrode. In this figure the model used in Group 3 in paper I is shown in a). The implant is seen in b) after cochlear implantation through a cochleostomy adjacent to the RW.

In the current papers, the dummy electrode lacked metallic stimulation electrodes, as the hypothesis was related to the insertion and presence of the electrode itself and not to electrical stimulation (Figure 6). This was also preferred as metal artefacts may disturb micro-CT imaging (paper II). The array was fixed inside the bulla and remained there during the entire study period, also after dissecting out the cohlea. In paper I it was made of polyethylene tubing and silicone and in paper II of a flexible and elastic silicone polymer.

Patients (Papers III and IV)

The studies in paper III and IV involve ten children with x-linked malformation resulting in inner ear malformation (Incomplete Partition type 3, IP 3). Their hearing loss was discovered at neonatal hearing screening in nine cases and after attempts at early hearing aid fitting, they were referred to the Cochlear Implant Clinic at the ENT Department at the Karolinska University Hospital. Further examination with temporal bone imaging and genetics revealed a *POU3F4* mutation explaining their mixed severe-profound hearing loss. They were deemed suitable for a cochlear implantation, and the mean age at first implant was 1.8 years (range=0.9-2.8 years). Five of these children received a second implant in a sequential procedure, and the others use a hearing aid on the contralateral ear.

As described above, this particular malformation includes severe modiolar dysplasia and the cribriform bone in the fundus of the cochlea is absent. This leads to an open communication to the internal auditory canal (IAC) and the cerebrospinal fluid (CSF) space. Opening to the intracochlear space results in a gusher of CSF that lasts for approximately 15 minutes. The properties of this specific malformation requires special procedures. First, intraoperative x-ray is used to confirm that the electrode array is positioned inside the cochlea and not displaced into the IAC. Second, the opening in the cochlea must be firmly sealed to avoid postoperative rhinorrhea. The surgical procedure developed at Karolinska University Hospital includes RW insertion and the use of a straight electrode array. It is described in detail in paper III.

In paper IV the study subjects with x-linked malformation were compared with a control group. The control group were children with Connexin 26 mutations resulting in deafness; they were recruited from a larger group of tested children and matched for gender, age and use of cochlear implants.

AUDIOLOGY

Objective Measures (Papers I and II)

Electrophysiological measurements allow hearing thresholds to be determined in patients or study animals without active participation of the subject. In the techniques described below, the electrical response from intracochlear potential or nerve impulses generated by multiple short sounds are recorded and averaged. This results in a graph with time on the x-axis and electrical potential on the y-axis. The sounds presented can be multi-frequency clicks, testing the whole organ of Corti, or a pure tone, testing the cochlear region corresponding to that frequency. A decreasing sound stimulus level gives a successively reduced electrical response and it is thereby possible to identify a threshold. In animal studies the thresholds for different frequencies are defined in every individual animal before any intervention.

Electrocochleography (ECochG)

Electrocochleography registers the electrical potential created inside the cochlea during sound stimulation. It reflects electro-mechanical processes in the organ of Corti and alterations in the response may therefore indicate the presence of endolymphatic hydrops (Fridberger *et al.*, 1997). In paper II, where possible hydrops after cochlear implantation was examined, ECochG was used at different time points after surgery (one day, one week, four weeks and three months). The anaesthetized animal was placed in a sound proof chamber with reference

electrodes on the scalp and hind leg (ground). After a postauricular incision and bullostomy, a gold ball recording electrode was placed in the RW niche. Sound stimuli were 8-ms tone bursts at the frequencies 2, 4, 8, 16 and 32 kHz, averaged over 125 stimulus presentations (Figure 7). In this study, cochlear implantation followed the first recording and in the final recording the bulla was opened but the cochlear implant kept in place.

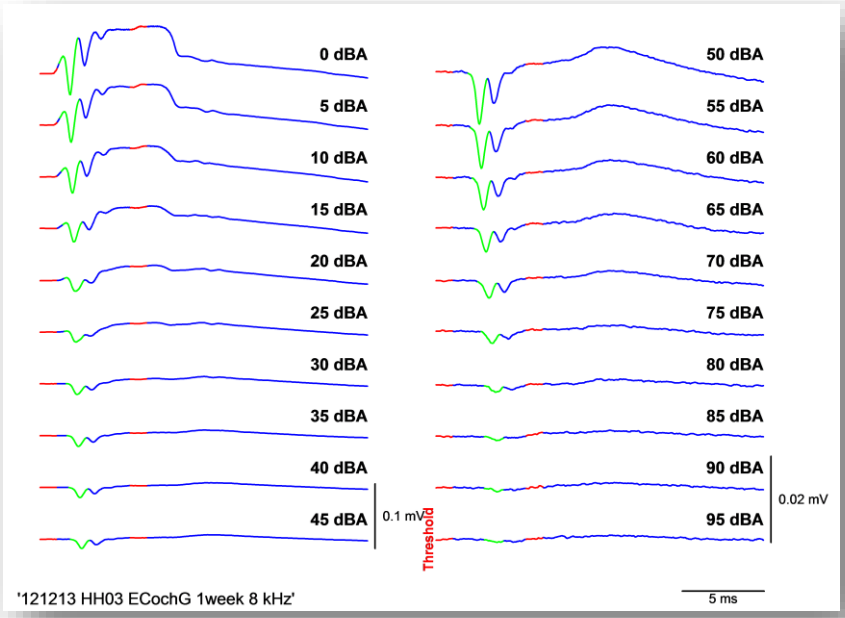

Figure 7) Example of electrocochleography recording, in this case in animal HH03 one week after cochlear implantation. Sound stimulation frequency is 8 kHz. The compound action potential (AP) is marked in green and the time period for measuring the summation potential (SP) as red. With increasing attenuation of the sound (sound intensity decreasing by 5 dB steps) the AP response decreases. Note the change in the response scale bar from 0.1 mV to 0.02 mV to the left (dBA, Decibel Attenuation).

Hearing threshold was defined as the lowest intensity that gave a response, compound action potential (CAP) of >0.5 µV. The first negative to the first positive peak indicated the CAP amplitude (green in figure 7). The SP was measured at 5.5-6.5 ms following onset of stimulus at the relatively stable plateau after the CAP (red in figure 7). A SP/AP ratio was calculated as a functional estimate of hydrops. Separately for each stimulus frequency postoperative SP/AP ratios were divided by pre-operative SP/AP ratios to quantify the change in the ratio over time. This gave an index, here called the "SP/AP ratio change" for each frequency.

Auditory Brainstem Response (ABR)

Auditory Brainstem Response (ABR) was used in paper I to evaluate electrophysiological hearing thresholds in the guinea pigs. The anaesthetized animal was placed in a sound proof box with electrodes attached to the vertex, the postauricular area and the hind hip. The loudspeaker was connected to the external auditory canal with a silicone tube. The initial sound stimulus was 90 dB peak SPL. A 5 dB reduction of the sound gives a successively reduced electrical response and the hearing threshold is estimated to when wave V, corresponding to

the synapse of the cochlear nucleus in the brain stem, disappears. The threshold is defined as the lowest intensity with a visible wave V in two averaged runs. Six different pure tone frequencies were analyzed in this work, 1, 2, 6.3, 12.5, 16 and 20 kHz. A postoperative *threshold shift* is calculated as the difference between the preoperative threshold and the measured threshold at different time points after surgery. I paper I these were immediately after insertion (0), day one (1), day four (4), one week (7), two weeks (14) and four weeks (28).

Psychoacoustic Methods (Papers III and IV)

Methods to determine hearing in subjects often depends on a response or interaction where the subject indicates that he or she has heard something, in adults and older children by pressing a button. These methods are called psychoacoustic. The tests are performed in sound-proofed rooms with low ambient sound level and short reverberation time.

Pure tone Audiometry

The most common way to test hearing is pure tone audiometry, which we often refer to as a "hearing test". This indicates a level of *detection* of sound at different frequencies. In the present studies, when the children were too young to interact by pressing a button to indicate when they hear, their responses were determined by observing their reactions to sound (behavioral observation audiometry) (Karikoski *et al.*, 1998)) or with visual reinforcement audiometry, (Shaw *et al.*, 2004). This pediatric audiometry requires an experienced child audiologist. In paper III and IV, children were tested with the sound presented by loudspeakers, in "free field" conditions (not with headphones). A crucial advantage of free field testing is that it allows hearing aids or cochlear implants to be used. Children between 2.5 and 5 usually are tested with conditioned play audiometry (Dawson *et al.*, 1998).

Speech Audiometry

Speech audiometry allows assessment of the level of word *discrimination*. Testing how subjects actually experience the words presented, by repeating what they just heard, permits better evaluation of how well their hearing works in everyday life. Words are composed by multiple frequencies, vowels and consonants, and word discrimination therefore requires a relatively well functioning cochlea, as well as central auditory pathways. This is referred to as *speech recognition*. In paper III and IV the children were tested with monosyllabic phonemically balanced word lists (Haskins, 1949, Liden, 1954). The results are presented as a percentage correct. The test was performed in quiet with the words presented at 65 dB SPL from a loud speaker in front of the child and in noise with the same speech signal but disturbed by a noise signal from four separated loud speakers (+/- 45-135 degrees azimuth). The signal-to-noise ratio was fixed at 0 dB. The mean age of the children tested in the x-linked group was 7.8 years (range=9.8-5.6, n=6). In the control group in paper IV the children had a mean age of 7.2 years (range=4.5-12.7, n=5). Children younger than four years cannot be tested with speech audiometry. The speech testing was performed with cochlear implants and/or hearing aids.

Localization of sound

Sound localization along the horizontal dimension requires two functioning ears. The ability to localize sounds is important, since it also facilitates the understanding of speech in noisy

situations (Hawley *et al.*, 2004). As binaural hearing is the aim in most children with severe-profound hearing loss, with cochlear implants or in combination by a CI and a hearing aid, they may develop spatial hearing. However, in spite of binaural stimulation, sound localization accuracy remains poorer in children with CI (Lovett *et al.*, 2010, Asp *et al.*, 2015). In paper III and IV, children were presented to sound from five loud speakers in a semi-circle separated by 45 degrees (spanning 180 degrees). The measurement consisted of 10 sound stimuli presented in random order from any of the loud speakers, and children were instructed to point at the loud speaker that generated the sound (Asp *et al.*, 2011). All tested children had binaural hearing, either with two cochlear implants or a cochlear implant in one ear combined with a hearing aid on the other ear (bimodal).

MORPHOLOGY (PAPERS I AND II)

After final electrophysiological measurements, the cochleae of the guinea pigs were prepared for histological analysis. The animals were euthanized with a pentobarbital sodium injection (25 mg/kg intraperitoneal) and either decapitated directly (paper I) or transcardially perfused with 0.9% saline followed by 10% neutral buffered formalin (paper II).

Surface Preparation

The cochleae were removed from the temporal bone and the perilymphatic space was perfused with 4% paraformaldehyde. The bony outer wall of the cochlea, the stria vascularis, Reissner´s membrane and the tectorial membrane were removed with forceps and the exposed organ of Corti was stained with Phalloidin to enhance the actin in the stereocilia of the hair cells. Following this, the basilar membrane was dissected from the osseous spiral ligament in half-turns and mounted on a microscope glass slide. The hair cells were examined in a fluorescence microscope (Zeiss) and missing inner or outer hair cells were counted, apex to base (Figure 8). The organ of Corti is 19 mm long in a guinea pig. Hair cell loss, seen as scar formation of the cuticular plate, were presented in place-specific cochleograms representing a percentage loss along the organ of Corti.

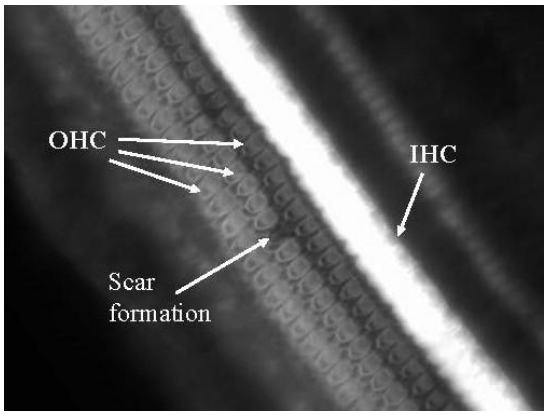

Figure 8) Picture of the organ of Corti in a guinea pig in a fluorescence microscope after surface preparation. The three rows of outer hair cells (OHC) are visible with one scar formation in the middle row. (IHC Inner Hair Cells)

Spiral ganglion Cell Density (Paper I)

The cochleae were removed from the temporal bone, trimmed and decalcified in 0.1M Ethylene-Diamine-Tetraacetic Acid for two weeks. Following dehydration, they were embedded in 2-hydroxyethyl methacrylate plastic (Technovit 7100) attempting an orientation for mid-modiolar sectioning. 24 µm cryosections were sliced and mounted on glass slides, keeping the electrode array within the cochlea. The nuclei of the spiral ganglion neurons were counted with an optical fractionator technique (Gundersen *et al.*, 1988), examining every tenth section with a 12 µm deep "optical dissector". An estimate of the total SGN number within the cochlea was calculated (Voie *et al.*, 1993).

Micro-CT (Paper II)

After collection of the cochleae, they were prepared for examining with micro-CT. The round window was incised, and a small opening near the helicotrema was made to facilitate staining of the intracochlear soft tissues with 4% osmium tetroxide. The cochleae were examined with an Xradia microXCT-200 scanner, aiming for a mid-modiolar axis of rotation (Figure 9). A reconstruction software, TXMReconstructor, was used to reformate a three-dimensional volume and, after corrections, slicing this for image analysis (Amira 5.4, Visualization Sciences Group, France). The presence of endolymphatic hydrops (EH) was identified by examining the position of Reissner's membrane (RM). The extent was quantified, at the positions along the basilar membrane corresponding to the frequencies examined with ECochG (2, 4, 8, 16 and 32 kHz).

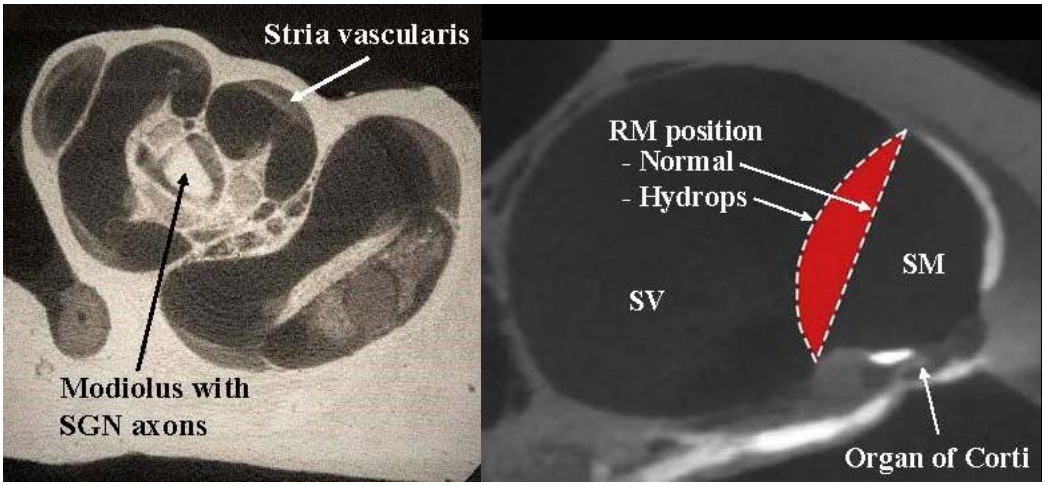

Figure 9) To the left a non-reformatted micro-CT image of a guinea pig cochlea. The stria vascularis, organ of Corti and Reissner´s membrane were visible. To the right a magnification of the basilar membrane, scala media and scala tympani. Marked in red is the method of calculating "scala media area differences" (SMADs) at each location along the cochlea. The SMAD is the area between the observed position of Reissner's membrane and its normal resting position. (SGN Spiral Ganglion Neuron, SV Scala Vestibuli, SM Scala Media)

Two methods to quantify EH was used. A developed Radiological Hydrops Score (RHS) was calculated by initial transmodiolar visual identification of the position of RM in relation to its neutral position. The displacement shape of RM was first defined by visual inspection as straight (normal), convex (hydropic), concave ("enhydropic") or flaccid, where RM was longer than usual, but its curvature was inconsistent. To arrive at a measure of EH the RHS (value 0-1) was calculated in every cochlea as the proportion of the RM that showed the convex shape typical of EH. For a second measure of EH was the "scala media area difference" (SMAD) was calculated (in %). The SMAD is the area between the observed position of RM and its normal resting position (Figure 9 right). SMAD was 0 at the normal position of RM. A convex RM, as in a hydropic cochlea, resulted in a positive SMAD value whereas a concave RM gave a negative value. For a summary measure for each cochlea the values obtained at the different positions were averaged.

The extent of soft tissue around the electrode was calculated by identifying the limits of scala tympani followed by coloring of the electrode on every fifth slice, interpolating the intervening slices (Matlab). Areas in the scala tympani with higher radio-opacity were identified as soft tissue. Several measures were quantified, the total tissue response volume, the tissue response volume isolated to each quadrant (defined as upper inner (Q1), upper outer (Q2), lower outer (Q3) and lower inner (Q4)) of the scala tympani, the maximum percentage of the cross-sectional area of scala tympani that was occluded by tissue response or electrode at any point along the cochlea, and the amount of tissue response within 200 µm of the organ of Corti..

GENETICS (PAPERS III AND IV)

A blood sample was collected from the participants for DNA extraction. Primary molecular testing for mutations in *POU3F4* was performed with multiplex ligation-dependent probe amplification (MLPA), which is a DNA-based method for detection of deletions and duplications (Schouten *et al.*, 2002). The mix of probes included two in the *POU3F4* gene as well as 4 probes targeting the 1 Mb regulatory region upstream of *POU3F4*. In addition, probes for other common exons involved in hearing loss were included such as all *GJB2*, *GJB6* (Connexin 26 and 30, DFNB1) and *GJB3* exons (Connexin 31) as well as *WFS1* (Wolfram syndrome) (P163-D1, MRC-Holland).

In cases were no mutation was found with MLPA, Sanger sequencing of POU3F4 was performed in order to detect point mutations in exon one of the gene.

SPOKEN LANGUAGE, COGNITION AND MENTAL HEALTH (PAPERS III AND IV)

Speech- and language pathologists assessed the speech and spoken language performance of each subject. A series of test materials were used aiming to recognize the specific difficulties for children with cochlear implants. Paper III focused on speech intelligibility and expressive grammar whereas paper IV also included verbal fluency, vocabulary and commutative aspects of pragmatic skills (Table 1). For assessment of cognition a psychologist evaluated the children with a series of tests and questionnaires focusing on executive functions. Mental health was assessed by questionnaires and focus group discussions.

Spoken language

For *expressive vocabulary* the Swedish version of the Boston Naming Test (BNT) was used. Stanine (STAndard NINE, scores following normal distribution divided into nine intervals) results are reported in relation to age (Tallberg, 2005). *Verbal fluency* was assessed by FAS-test (testing phonemic fluency on the letters F, A, S) and Animal (testing semantic fluency). *Pragmatics*, rules for social language, were evaluated by the Children's Communication Checklist-2 edition (CCC-2) (Bishop, 2003) parental screening questionnaire estimating a child's communication skills. (In addition to pragmatics it assesses the areas of syntax, morphology, semantics, and speech.) Different subscores such as General Communication Composite score (GCC) or Social Interaction Difference Index (SIDI) may be obtained identifying weaknesses in different areas of language. In the area of pragmatics the scores relate to nonverbal communication, social relations and interests. To test how comprehensible the participants' speech was the Speech *Intelligibility* Rating-2 (SIR-2, score 1-5) (Allen *et al.*, 2001) was used and rating of *expressive grammar* was done by a speech-language pathologist using a locally developed scale (EGS, score 1-9) (Löfkvist, 2014).

Table 1) Tests of spoken language, cognition and mental health used in papers III-IV.

Measure/Test name	Paper III	Paper IV
Spoken language ability		
Expressive vocabulary; The Boston Naming Test (BNT)		X
Phonemic word fluency task; (FAS)		X
Semantic word fluency task; (Animal)		X
Pragmatic skills; Children's Communication Checklist-2 edition (CCC-2)		X
Speech intelligibility; Speech Intelligibility Rating-2 (SIR-2) (rating of speech-language pathologist)	X	X
Expressive grammar; Expressive Grammar Scale (EGS) (rating of speech-language pathologist)	X	X
Cognition and mental health		
Executive functioning: Test of Everyday Attention for Children (TEA-Ch)		X
General Working memory; Sound Information Processing System (SIPS)		X
Phonological Working memory; SIPS		X
BRIEF-P and BRIEF (parent and teacher questionnaire)		X
Non-verbal cognitive ability (Ravens Colored Progressive Matrices)		X
Strengths and Difficulties Questionnaire (SDQ) (parent and teacher questionnaire)	X	X

Cognition

Evaluation of cognition focused on executive functions. In this sense cognition relates to working memory, attentional and inhibitory control, flexibility, as well as reasoning, problem solving, and planning. In this study, children older than six years were evaluated by the Test of Everyday Attention for Children (TEA-Ch, nine subtests) (Baron, 2001, Heaton, 2012). Their general working memory was assessed by the Sound Information Processing System (SIPS), the phonological working memory by the subtest Sentence Completion and Recall task (Wass, 2009). Questionnaires used were BRIEF (Behavior Rating Inventory of Executive Function, parents and teachers) (Isquith *et al.*, 2004) for judging executive functions and Ravens Colored Progressive Matrices for measuring abstract reasoning and intelligence (Raven, 2003).

Mental health

The mental health status of the children was measured by questionnaires and focus group discussions lead by a social worker. The standardized Strength and Difficulties Questionnaire, SDQ (Goodman, 1997, Goodman, 2005), was answered by the parents individually and, for the children in a school setting, by their teachers. The questionnaire ask about 25 attributes. Some of them are positive, others negative. They are divided into five scales consisting of *emotional symptoms*, *conduct problems*, *hyperactivity–inattention*, *peer problems*, and *prosocial behavior*. Each scale has five items scoring 0-2, generating a scale score of 0-10. The prosocial behavior scale is inverted compared to the others with lower scores indicates difficulties. The four first scales are added to a *total difficulty* score (0-40).

STATISTICS

In paper I and II, analysis of variance of data parameters from ABR or ECochG was performed with repeated measures ANOVA (Paper I used the software R for statistical calculations, and paper II used SPSS) and validated by Greenhouse-Geissner correction and, as with post-hoc testing, least significant difference (LSD) method for multiple comparisons (paper II).

Descriptive statistics were used to summarize or quantitatively describe features. For non-parametric comparison of medians analysis, Independent Samples Kruskal-Wallis (multiple groups, Paper II) and Mann-Whitney U-test (two groups, paper IV) was used.

RESULTS

PAPER I

Paper I is an experimental study on guinea pigs aiming to investigate cochleostomy and insertion trauma at cochlear implantation. This is done by examining alterations in hearing thresholds after surgery as well as intracochlear histological changes. Three groups of normal-hearing guinea pigs underwent cochlear implant surgery. In group 1, only a cochleostomy (C) was performed, without insertion of an electrode, in group 2, deep insertion (DI) to a depth of 4.5 mm was used and in group 3 a "GP modified" insertion to a depth of 3.25 mm was performed.

Residual hearing

Hearing testing in the groups 1 and 2, followed for 14 days, revealed a statistical difference in hearing outcome seen already the first day after surgery (Figure 10). In both groups an increased threshold shift is noted after the first week, in group 2 this remains unchanged (indicating a permanent hearing loss). In group 1 however, the threshold shift normalizes during the second week to a 5-8 dB level on the four frequencies measured. In group 3 additional testing time points was added at 0, 4 and 28 days. In addition two lower frequencies tested were added, 2 and 4 kHz. For the three lower frequencies 2, 4 and 6.3 the pattern of threshold shift here shows clear similarities with group 1(C) with hearing loss remaining during the first week. On the other hand, the two higher frequencies tested, 16 and 20 kHz, showed initial similarities with group 2 (DI) with large initial threshold shift but with the difference of rapid decline indicating recovery of auditory function. After two weeks the thresholds had stabilized at a level of -1-10 dB remaining unchanged at four weeks. The threshold shifts at two weeks are significantly different to group 2 but not to group 1.

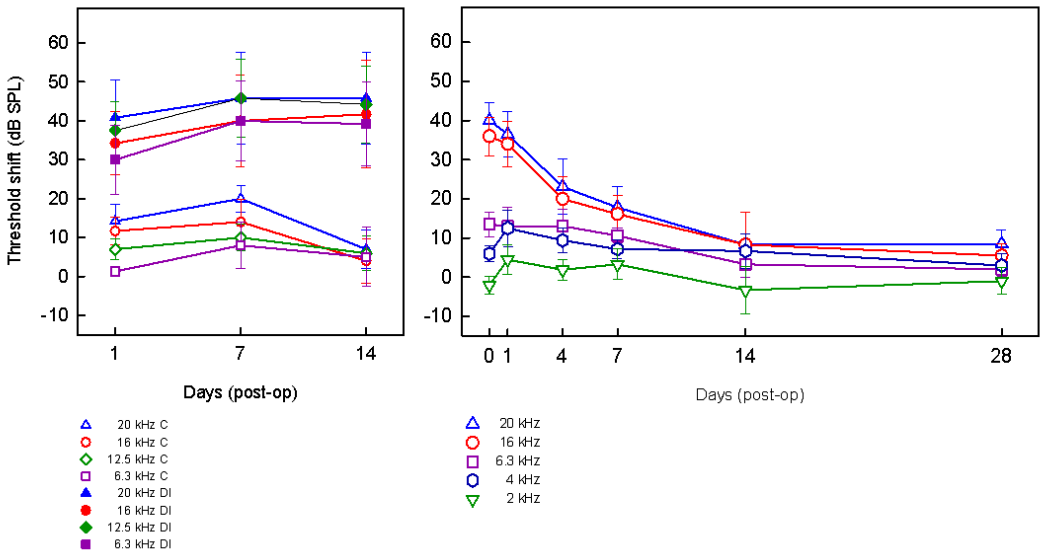

Figure 10) Hearing threshold shifts after surgery in guinea pig. Left graph with ABR results after cochleostomy alone (C) or deep insertion (DI) in the different frequencies respectively. Bars indicate SEM.

Histology

Surface preparation and plastic embedded sectioning was performed in the cochleae of group 3, five cochleae for each method. Examination showed very limited signs of trauma. Surface preparation cochleograms revealed close to no hair cell loss in three examined cochleae and 40-50% OHC loss in the most apical region (15-19 mm) in two cochleae. Threshold shifts in these two animals were not above average for the group. Sectioning revealed histology with very limited soft tissue reaction in three of five cochleae and more extensive in the remaining two. Hearing results in the corresponding two animals were below average. Spiral ganglion neuron (SGN) density did not differ from the result of non-implanted, untreated cochleae.

PAPER II

In an experimental setup with four groups of normal hearing guinea pigs the hypothesis of endolymphatic hydrops induced by cochlear implantation was studied. The four groups were followed for 1, 7, 28 or 72 days respectively.

Electrocochleography

Measurements were performed prior to surgery, immediately after cochlear implantation and at the end of the survival period in each animal. Compound Action Potential (CAP) and Summation Potential (SP) was recorded at the frequencies 2, 4, 8, 16 and 32 kHz.

At the end of the survival period the SP/AP ratio change increased relative to pre-operative levels at 1 and 7 days after surgery (Figure 11). This was rarely seen at 28 or 72 days.

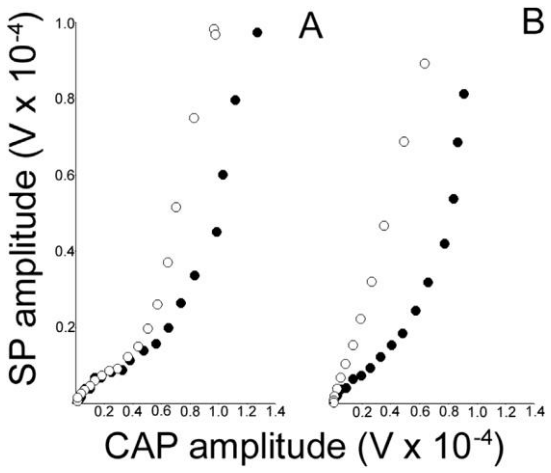

Figure 11) The summating potential (SP) amplitude plotted against the compound action potential (CAP) amplitude in response to a 16 kHz tone, both prior to implantation (solid marker) and at the experimental end point (open marker). Each point reflects data in response to one stimulus intensity. A and B are examples in two different animals 7 days after CI surgery. As stimulus intensity increases, both the SP and the CAP grow in magnitude. At 7 days after CI, the SP grows more rapidly than the CAP (as reflected by a steeper growth response), indicating that the SP/AP ratio is greater than pre-operatively.

Alterations in hearing, calculated as CAP threshold shifts comparing results before CI surgery, immediately after and at the end of the survival period showed large differences in the measured frequencies. The greatest hearing loss was seen in 8 and 16 kHz and immediately after implantation. At the end of experiment the loss was largest in the day 1 group with an average of 24.7 dB deterioration. The later groups showed less hearing loss, 7 days 6.4 dB, 28 days 5.2 dB and 72 days 15.6 dB.

Micro-CT

All implanted electrodes (21) were located within the scala tympani. There was no sign of fracture to the basilar membrane and only one fracture of the osseous spiral lamina. The Radiological Hydrops Score (RHS), calculated as an average of the examined cochleae, showed a significant hydrops at week 1 but not at day 1 and a tendency of less hydrops at later time points as compared to the contralateral ear (Figure 12). The SMAD, averaged across the cochlea, was significantly higher at day 1, 7 and 28 after implant surgery than at 72 days.

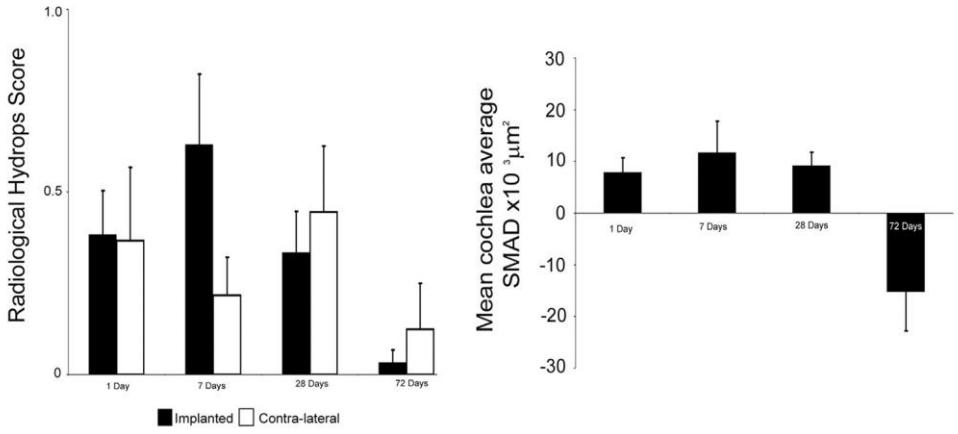

Figure 12) To the left the Radiological Hydrops Score (RHS) after cochlear implantation as an average of hydrops in the four groups of guinea pigs. To the right change in SMAD (scala media area difference) in the same groups.

The soft tissue response calculated around the electrode in the basal turn had an average volume of 0.93 mm^3 representing a median occlusion of the scala tympani of 59% (Figure 13). Most of the tissue response was located in the upper outer quadrant (Q2) of scala tympani (51.2%). There was only a small non-significant difference in the soft tissue response in the four groups.

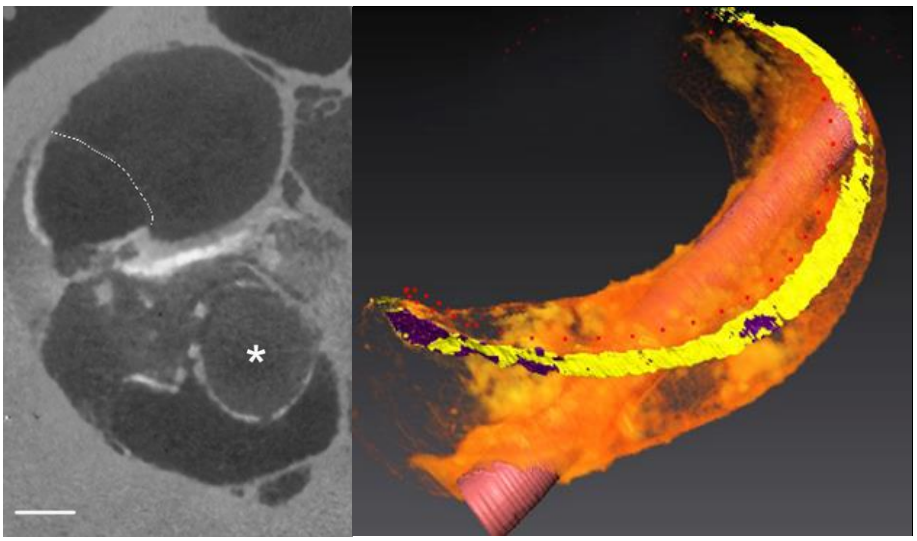

Figure 13) To the left a micro-CT with electrode array (*) and tissue reaction within scala tympani in the basal turn. RM marked white indicating hydrops. Bar equals 150 mm. Right figure is a reconstruction of the basal turn. The implanted electrode array is seen in red in the scala tympani. Yellow is the organ of Corti and orange tissue response around the electrode.

PAPER III

In this clinical study a group of ten children, nine boys and one girl, with x-linked malformation were evaluated in a retrospective chart review in combination with an assessment day for additional auditory and speech and language testing. They had been referred for CI assessment and were seen in clinic at an average age of 1.4 years. They all received a first cochlear implant at an average age of 1.8 years (range=0.9-2.8 years). Subsequently five received a second implant on the opposite ear at average 2.7 years of age (range=2.0-3.8 years).

Cochlear implantation

Fifteen implantations have been performed with an average follow-up time of 4.2 years (range 0.1-8.1 years). All implants were provided by MED-EL®. The electrodes used were straight with, in thirteen cases, a length of 24 mm. In one case a 28 mm electrode was used and in another a 31 mm electrode. In all cases a gusher was encountered. On insertion the electrode deviated into the internal auditory canal in three cases, revealed on intraoperative x-ray (Figure 14). Access to scala tympani was obtained through the round window in twelve cases and by cochleostomy in three. Firm packing around the electrode sealed the opening. In four cases 2-3 electrodes could not be fully inserted, one of those cases being the 31 mm electrode. One case, the first, developed a postoperative rhinorrhea present for six days but ceased on conservative treatment.

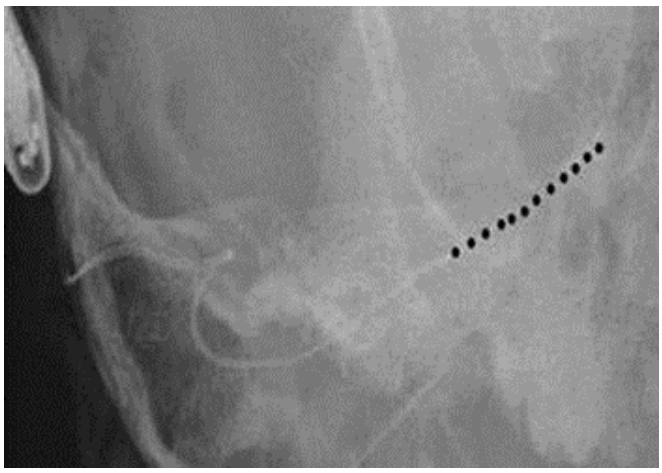

Figure 14) Electrode array seen incorrectly positioned in the IAC where the electrodes on the array are highlighted by black dots.

Audiological outcome and communication

All children have binaural stimulation, five with a contralateral hearing aid. The six oldest children were able to perform speech and localization testing. Speech recognition was significantly higher in the mean obtained at the national two-center study (nat2) of pediatric CI recipients than in the x- linked-group in both quiet and noisy conditions (mean %+/-SD) (x-linked 48+/-19 versus nat2 87+/-16 in quiet and 24+/-10 versus 61+/-20 in noise). Sound localization accuracy was significantly better than chance performance in five of the six tested subjects (Error Index 0.36+/-0.14. Four of the tested children had bilateral CIs, the other two hearing aids).

All children use their CIs full time and all have developed spoken language. The three oldest use only spoken language whereas the remaining are either bilingual (three of whom two are brothers with a deaf father) or use supported signs (the younger children and one child with additional cognitive handicap).

PAPER IV

This paper involves the same ten children as in paper III (x01-x10). These children with cochlear implants have shown similarities in their behavior in clinic at follow-up, indicating problems beyond their hearing loss. The study therefore focuses on their genetics and outcome regarding spoken language, cognition and mental health compared to a control group of pediatric CI recipients without cochlear malformation (Connexin 26 (cx 26)). Their mean age at last follow-up was 6.0 years (range=2.0-9.7) (cx26 6.6 years, range=1.1-14.6) with a total CI follow- up period of 4.2 years (range=1.1-8.1) (cx26 4.7 years, range 0.4-11.1). The mean age for first implant was similar in both groups (x-linked 1.1 years (range=0.9-2.8), cx26 1.9 years (range=0.6-5.5)). A large variability is seen in audiological outcome across groups when testing speech in quiet and speech in noise. Speech recognition was significantly higher in the cx26-group than in the x- linked-group in both quiet and noisy conditions (mean %+/-SD) (x-linked 48+/-19 versus cx26 82+/-17 in quiet and 24+/-10 versus 53+/-15 in noise).

Genetics

Six of the children (x01, x04, x05, x06, x07 and x10) had previously been involved in genetic testing verifying their mutations in the *POU3F4* gene and upstream region (Figure 15). Additional testing revealed mutations in another three children. One of the children is a girl (x07) with a very large deletion in the region of the gene. This child has additional handicaps including motor skills and more severe cognition deficits and analysis for x-linked inactivation is pending. Two previously undescribed point mutations were found (x08 and x09).

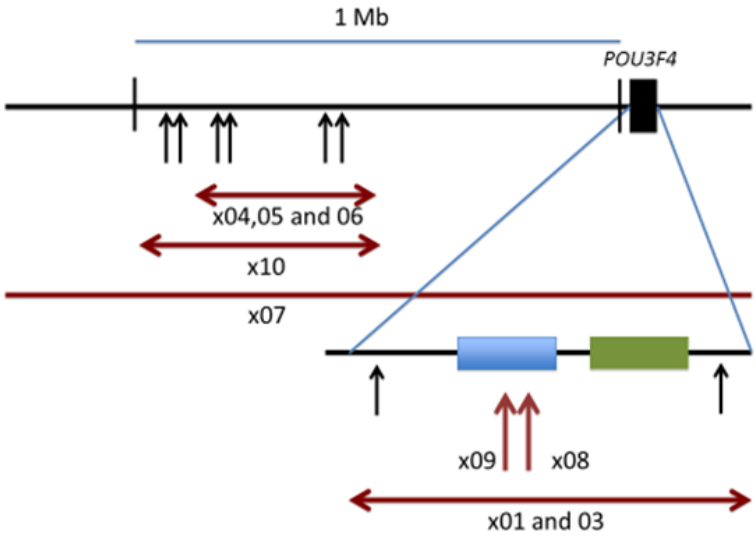

Figure 15) Location of POU3F4 mutations. The green bow represents the POU domain and the blue box the POU-specific domain. Horizontal red bars represent minimal region of deletions. Vertical red bars indicate the location of the point mutations. Vertical black bars represent the location of the MLPA probes.

Spoken language

The x-linked group scored significantly poorer in *expressive vocabulary* in the Boston Naming Test (BNT, stanine score) between groups (x-linked versus cx 26 z= -2.1, p=0.035) and on raw scores (z= -2.31, p=0.021). *Pragmatic skills* reported from parent questionnaires (CCC-2) showed three areas of significant difference with poorer performance in the x-linked group; total score (z= 2.41, p=0.015) and on the subscales coherence (z= -2.31, p=0.011) and context (z= -2.74, p=0.015). A significant difference was also found for speech *intelligibility* rating (SIR2) (z= -2.30, p<0.014) No significant differences were found between groups in word fluency tasks (FAS and Animal) or expressive grammar (EGS).

Cognition

Only four children in the x-linked group completed the TEA-Ch test due to fatigue or poor concentration. Results revealed a significant difference in only one of the subscales, Spacehunt-TIME (Z= -2.34, p=0.019). Some group-specific differences were seen with the BRIEF parental and teacher report namely, Global Executive Composite of executive functions

(parents z= -2.31, p<0.021, teachers z= -2.17, p<0.030) and Behavior Regulation Index (parents z=- 2.25, p<0.025, teachers z= -2, 01, p<0.045). On three subscales for parents significant differences were found; Emotional control (z=-3.01, p<0.003), Initiate (z=-2.17, p<0.030) and working memory (z=-2.08, p<0.038). On the general working memory task (Sentence Completion and Recall) the children with x-linked malformation had significantly lower scores than controls on the phonological working memory task (Serial recall of non-sense word-2, z= - 2.08, p<0.037) but not on total scores (p>0.1). However, the group of children with x-linked malformation revealed a difference with more semantic irrelevant responses. No statistical significant differences were found between groups in Ravens Colored Progressive Matrices measuring non-verbal cognitive ability (x- linked vs. cx26) (p's>0.1).

Mental Health

All mothers and almost all fathers of the x-linked group rated the children in a way that indicated mental ill-health (x-linked 15.75 vs. cx26 6.2, p<0.001) on the total difficulties score (total sum of scales 1-4). Further, parents of children in the x-linked group reported significantly higher scores compared to the cx26 group on emotional symptoms (2.33 vs 1.27, p=0.028), conduct problems (3.50 vs. 1.27, p=0.003), hyperactivity-inattention (8.17 vs. 2.60, p<0.001) and impact score (3 vs 0, p<0.001), and lower on prosocial behavior (7.67 vs. 8.93, p=0.012) Teachers scored x-linked significantly higher on hyperactivity-inattention (7.5 vs. 2.71, p=0.035), peer problems (1.83 vs. 0.43, p=0.022) and impact score (3.40 vs. 0.17, p=0.033), and lower on prosocial behavior (4.5 vs. 8.29, p=0.022). Seven of the children attend mainstream school, two go to special units and one attends a deaf preschool.

DISCUSSION

This thesis discusses aspects of cochlear implantation in two specific groups of patients. Both groups, patients with partial deafness and pediatric cochlear implant candidates with a malformed inner ear, constitutes an increasing part of the patient population at the Cochlear Implant Clinic at Karolinska University Hospital. For both patient categories individualized treatment is of vital importance for the outcome. Every cochlear implant candidate should expect a treatment with a combination of an intervention with minimal surgical trauma and a maximum of benefit in relation to each individual's condition and cause of hearing loss. For the two groups in this thesis this is clearly visible. Individualized follow-up in a team of experienced audiologists and engineers as well as trained speech-and language pathologists and social workers is also essential for both patient categories.

HEARING PRESERVATION DURING COCHLEAR IMPLANTATION

The surgical technique for hearing preservation in a cochlear implantation procedure is standardized, not differing much from a routine CI case, and described by several authors (Lenarz *et al.*, 2006, Lorens *et al.*, 2008, Van Abel *et al.*, 2015). A transmastoid-facial recess approach, taking care not to drill on the head of the incus or manipulate the ossicular chain, is used. Opening of the cochlea may be performed by a cochleostomy, carefully drilling to visualize the endosteum but not entering the scala tympani. However, a RW approach is preferred by most surgeons. For this, the RW bony niche is removed in order to visualize the RW membrane. At this point, the middle ear is rinsed and corticosteroids applied on the RW membrane or the endosteum. The choice of steroid has differed, but often Triamcinolone is used. Some surgeons prefer to give one dose of iv steroid, often Hydrocortisone or Betamethasone, prior to opening the cochlea. An incision is made in the RW membrane or endosteum and a thin and flexible electrode array slowly inserted. Straight arrays are preferred by most authors, and have shown good hearing preservation results. Care is taken to stabilize the electrode and avoid suctioning near the opening of the cochlea. The seal of the cochlea is softly applied and not packed around the array. In addition to the corticosteroids given during the procedure, some authors recommend one week of oral methylprednisolone or prednisolone.

Paper I demonstrates that deep insertion (4.5 mm, group 2), with the associated large trauma, results in a substantial threshold shift, concluding that the insertion in this case alone is responsible for the permanent hearing loss. This most likely happens because rupture of the basilar membrane leads to a loss of cochlear potentials. In hearing preservation cases a deeper insertion than 360° is not likely to be beneficial, even though there have been reports showing residual hearing preservation with longer electrodes (Tamir *et al.*, 2012, Nordfalk *et al.*, 2016).

The benefit of corticosteroids for hearing preservation has been demonstrated in animal studies (Chang *et al.*, 2009, Maini *et al.*, 2009, Connolly *et al.*, 2011, Lee *et al.*, 2013). There are also indications that an extended delivery of steroids, as with the postoperative regime in humans, may benefit hearing preservation (Rah *et al.*, 2016). Clinical studies regarding possible benefits of steroid treatment are underway (Enticott *et al.*, 2011).

Several authors have demonstrated that hearing preservation can be achieved. This is clearly desirable, as there is evidence that a combined electrical and acoustic stimulation is beneficial,

improving hearing, especially speech in noise (Gstoettner *et al.*, 2009, Kong *et al.*, 2015), and gives improved quality of sound and music (Brockmeier *et al.*, 2010). In a meta-analysis of 24 studies Santa Maria and co-authors concluded that cochleostomy was associated with better hearing preservation as compared to the round window approach. Also a slow electrode array insertion technique was superior to insertion of less than 30 seconds and the use of postoperative systemic steroids (Santa Maria *et al.*, 2014).

However, most studies examine short term results. Long term clinical studies reveal a trend of slow and continuous hearing loss. This may relate to the pathology of the individual reason for hearing loss, thus, the patient did not have a stable hearing loss prior to surgery, but is in most cases likely to depend on an intracochlear process leading to hearing deterioration. The nine subjects Mertens and colleagues (Mertens *et al.*, 2014) followed for up to eleven years a gradual loss was seen for most of them. Erixon and co-workers (Erixon *et al.*, 2015) followed 19 patients for up to three years, displaying a gradual low frequency loss and recently Helbig *et al*. reported on a large number of patients (n=96) that were followed up to eleven years (Helbig *et al.*, 2016). Here a similar pattern of gradual loss is seen, however with large individual variability. The loss seen exceeds that of the contralateral ear and these studies clearly show that there is an ongoing intracochlear process that must be explained by the implantation itself.

LOSS OF RESIDUAL HEARING AFTER COCHLEAR IMPLANTATION

The most important finding in paper I is that there is no statistical difference (p=0.13) between performing a cochleostomy (group 1) compared to combining this with a moderate insertion (group 3). From this perspective, the electrode insertion itself does not explain the alterations in hearing seen in the early postoperative period. In guinea pig cochlear implantation, other authors have used insertion depths ranging from 2.25-3.25 mm (Braun *et al.*, 2011, Connolly *et al.*, 2011, Quesnel *et al.*, 2011). For these moderate, less traumatic, insertion depths the authors have been able to preserve hearing, often in combination with corticosteroid treatments.

Histopathology of patients with a CI reveals damage to intracochlear structures like the spiral ligament, stria vascularis and hair cells in most implanted ears (Fayad *et al.*, 2006). In a human cochlea it is shown that the force necessary for insertion increases beyond 20 mm, when a straight electrode is used (Adunka *et al.*, 2006). Therefore, a moderate, less traumatic, insertion in a human cochlea could be approximated to 20 mm, corresponding to a distance near 360° in most cochleae. However, this varies since the length of the basal turn lateral wall ranges from 20.7 to 24.2 mm (mean 22.8 mm; (Erixon *et al.*, 2009). Very shallow insertion (8 mm), with a lesser cochlear coverage, leads to worse speech perception results (Buchman *et al.*, 2014). An insertion of 20 mm insertion is shown to give an adequate auditory outcome in a study by Adunka and colleagues in 2010 (Adunka *et al.*, 2010). Here they could demonstrate that a 20 mm electrode (where the tip approximately reaches the region of 1000 Hz on the organ of Corti) gives speech perception performance comparable to a longer electrode (31.5 mm).

As there is controversy regarding the possible benefits of deep insertion (Boyd, 2011) one could argue that a 360° insertion should be attempted in all cases of implantation as it will give a reasonably good auditory outcome while minimizing trauma. Minimizing intracochlear damage is important for future cochlear implant revision surgery or other, today unknown, alternative intracochlear treatments. On the other hand, there are indications that a deeper

insertion is beneficial to improve speech understanding in noise as well as the perceived sound quality (Hochmair *et al.*, 2015). In cases of hearing preservation this is not an issue as the apical region is functioning with residual low frequency hearing and it is therefore not necessary to attempt to stimulate this region in any case.

Possible explanations for short- and long term permanent hearing loss have included a direct effect of the inserted electrode, structural changes and inflammation. Induction of pathways leading to apoptosis has also been suggested (Eshraghi *et al.*, 2006). The effects of a direct trauma, such as perforation of the basilar membrane, and leakage of fluids between compartments is an obvious acute reason for permanent hearing loss. Additional direct effects could come from the electrode volume itself creating altered cochlear michromechanical properties, leading to an "cochlear conductive component" (Banakis Hartl *et al.*, 2016). The electrode may also interfere directly with surrounding structures in scala tymapni and thereby affect the production, and absorption, of cochlear fluids. Further, inflammation caused by the surgical trauma and the presence of a foreign body has been suggested as mechanism for gradual sensorineural hearing loss. The inflammation may influence ion regulation and water permeability, possibly by altering vascular permeability in stria vasculris. This likely inflammatory process is the rationale for the use of corticosteroids. The inflammation may also induce development of soft tissues around the electrode array seen as fibrosis in histopathological examination.

In addition to structural damage, histopathological studies of human temporal bones have indicated presence of endolymphatic hydrops in patients with a cochlear implant (Handzel *et al.*, 2006, Richard *et al.*, 2012). Similar finding have been made in guinea pigs at three months following cochlear implantation (Lee *et al.*, 2013). Paper II tested the hypothesis that cochlear implantation may induce endolymphatic hydrops and that this could explain the gradual hearing loss seen in patients with residual hearing. The rationale for this was that long-term, fluctuating, endolymphatic hydrops is associated with permanent sensorineural hearing loss in combination with the finding of hydrops in cochleae with a cochlear implant. In addition, it is well known that patients may experience dizziness after cochlear implantation. The results show that hydrops is present during the first week after cochlear implantation but not later in the test period (28 and 72 days). Possibly this finding could explain the temporary threshold shifts seen in paper I with a large shift in ABR thresholds immediately after cochleostomy or a moderate implantation followed by a gradual normalization. A pattern that resemble the CAP threshold shifts in paper II.

MALFORMATIONS OF THE INNER EAR

During the last decades the knowledge of inner ear malformations have broadened, much related to better imaging techniques. As both CT and MRI today provide pictures with better resolution, more details may be revealed. The Jackler classification from 1987 was based on the polytomography technique with limitations in resolution (Jackler *et al.*, 1987). With increasing interest in cochlear malformations related to growth of pediatric cochlear implantation programs, Sennaroğlu and Saatci suggested a new classification in 2002 with the main contribution of further subclassifying the incomplete partition as type 1 and type 2 (Sennaroglu *et al.*, 2002). In 2006 the classification was extended with incomplete partition type 3 (Sennaroglu *et al.*, 2006) and is today widely accepted. In short this classification today

include *Michel aplasia* and *cochlear aplasia*, quite rare types, which both entail complete absence of the cochlea. Additional severe malformations include cystic vestibulo-cochlear malformations such as *common cavity* and *incomplete partition type1* (IP-1) and *incomplete partition type 3* (IP-3). The most common malformation is the less severe *incomplete partition type 2* (IP-2), with a well-shaped basal part and a moderate modiolar dysplasia including a defective apical region. The severity of *cochlear hypoplasia* (CH, cochlea less than normal size) malformations varies. Sennaroğlu subclassifies these into four groups, *CH type 1* with a cystic budlike appearance, *CH type 2* with a more normal external shape but still cystic or limited modiolar development, *CH type 3* where the cochlear duct is shorter than normal but with otherwise well-organized intracochlear structures and *CH type 4* with normal basal turn but dysplasic apex. Phelps (Phelps, 1992), Zheng and colleagues (Zheng *et al.*, 2002), Papsin (Papsin, 2005) and Giesemann and co-workers (Giesemann *et al.*, 2011) and have all suggested minor alterations in this classification.

In addition to cochlear malformation, the deformities of the vestibular part of the inner ear, the vestibule and semicircular canals (SCC), varies greatly from severe cystic malformations to mild SCC deviations. SCC dysplasia seem to be more common with hypoplastic cochlea malformations, as in cases with narrow IAC. The vestibular aqueduct may be enlarged (LVA) whereas the cochlear aqueduct seems to be very consistent. Theoretically a wide cochlear aqueduct, which establish a communication between the intracochlear perilymphatic space and the subarachnoid space, could give a gusher during surgery but this has never been encountered in the author's experience. LVA may exist alone or, as it often is, together with IP-2. It is very likely that with better future imaging resolution additional minor modiolar dysplasia will be discovered in the cases of LVA that today appear to have a normal cochlea

The possibility for histological examination of malformed human cochleae is limited. In a recent work, Sennaroğlu reports on the examination of 33 specimens in the collection of the Otopathology Laboratory at Harvard University's Massachusetts Eye and Ear Infirmary (Sennaroglu, 2016). They displayed a variety of inner ear malformations and he compared the results with CT and MRI images. He suggests that for cochlear hypoplasia the membranous labyrinth development determines the size of the bony cochlea. For severe internal architecture deformities, like in IP-1 and severe hypoplasia (CH-1 and CH-2), a deranged vascular supply from the internal auditory canal is the major contributing factor. For IP-2 an enlarged endolymphatic sac (with LVA) is likely to be responsible for the modiolar dysplasia related to high intracochlear endolymphatic pressure. There was no specimen with an IP-3 malformation in the collection but the author suggests that the thick inner endosteal layer of the otic capsule forms the abnormal shape, when related to the thin or absent middle endochondral and outer periosteal layers of the surrounding bone. In IP-3 the organization of the membranous labyrinth and spiral ganglion neurons remains unclear as the modiolus is completely absent. As the cribriform base of the cochlea is missing there is an open connection to the CSF space.

Jackler (Jackler *et al.*, 1987) suggested a developmental arrest theory for the different malformations. Interruption during inner ear embryogenesis were to result in the different cochlear dysplasia's seen, most of them around the sixth gestational week. The cochlear duct develops during a remarkably short period, only sixteen days from a cochlear pouch to its full length (Streeter, 1949). For cochlear aplasia a developmental arrest may explain the morphology but for other types, the linear Jackler arrest theory has to be regarded with some

skepticism. With the existing knowledge from CT and MRI in combination with the above described histopathological findings it is more likely a multi-factorial course of events after the cochlear bud development. It is likely that multiple, independent, arrests may occur for the differences in development of size itself and internal structures. These paths do not seem strictly related as all types of combinations may be seen. The work by Papsin 2005 suggested a classification of cochleovestibular anomalies based on independent arrests of development.

Known genetic factors causing inner ear malformations are *POU3F4* mutation in x-linked malformation and *SLC26A4*, encoding for the Pendrin protein (Pendred syndrome) with the associated IP-2 malformation. There is likely to be other, today unknown, gene mutations or combinations of these, responsible for the other types of malformations we see. Although genetics is likely to be the primary reason for anomalous development, environmental factors, such as for instance viral agents, may also play a part. The fact that a malformation may be unilateral or that a patient may have malformations of different severity on either side indicate multifactorial etiologies.

Classification of a condition is important for several reasons. The subtypes may be studied relating to challenges during surgery, identifying pit falls, thereby ensuring that the surgeon is properly prepared, minimizing risks for complications. Furthermore, by dividing cases into groups, the outcomes may be discussed in relation to specific malformations, which gives a possibility to identify special needs for follow-up and training. For anyone who sees many children with different cochlear malformations in clinic, it is obvious that patients differ greatly in audiological outcome and spoken language performance.

In our material of bony cochlear malformations we currently have 4% IP-1, 60% IP-2 and 15% with IP-3. In addition 21% of the cases have hypoplastic cochleae. The proportion of IP-3 is high compared to other data (Papsin, 2005, Sennaroglu, 2010). As described earlier, this malformation has a cork screw appearance of the cochlea, modiolar aplasia, absent cribriform bone in the fundus, and a wide internal auditory canal. The children in study III and IV have this type of malformation. The fact that these children have residual hearing indicate that there is some organization of the organ of Corti in spite the complete absence of the modiolus. The functioning hair cells have to be structured in a way where they still are susceptible to sound-evoked vibrations, and there has to be an intact endolymphatic space as well as a reasonable cochlear potential (normally a positive potential of 80-100mV). Further the spiral ganglion neurons have to be organized with the dendrites connected to the inner hair cells. Figure 3, page 7, shows bone-conduction thresholds ranging from less than 30 dB at 250 Hz, dropping to 65dB at 1 kHz and above. Air conduction thresholds confirm a very large air-bone gap (ABG). Hypothetically several factors can explain this ABG. A true conductive portion of the hearing loss such as stapes fixation is likely to play a role. In the author's experience, stapes fixation is often found in these patients during cochlear implant surgery, but improvements in mobility have been described once the high intracochlear pressure is relieved by opening the cochlea, hence, no true structural fixation would exist. Further, the fact that the membranous labyrinth to some extent has to be floating loosely, as the bony modiolar structures are missing, must radically change the mechanical properties of the organ of Corti. The shape of the audiogram in these patients show that the low-frequency regions of the organ of Corti retains more function. This may indicate a loss of the normal stiffness gradient of the basilar membrane (von Békésy, 1960). Other hypothetical reasons include non-functioning outer hair cells, which

would be consistent with a loss near 60 dB, or a high intracochlear pressure that interferes with the micromechanical properties of the organ of Corti. It has also been considered an option that the IP-3 has a false ABG related to an "inner ear conductive hearing loss" (Snik *et al.,* 1995). Further, if the opening to the IAC acts as a third window, the bone thresholds could be falsely elevated as the cranial cavity would be "acting as an amplifier" for bone-conducted sounds (Minor, 2003).

SURGERY TO THE MALFORMED COCHLEA

Early intervention is the aim for all treatment of congenital hearing loss. Neonatal screening is today a routine procedure in all developed countries, often with otoacoustic emissions. Further investigation includes ABR and ASSR. When hearing loss is found, the child is fitted with hearing aids at the earliest age possible. In severe-profound deafness, a MRI is performed, thereby visualizing the inner ear anatomy and auditory nerves. To administer this in the first four-five months of life, a strict routine needs to be followed.

With signs of inner ear malformation, a CT is performed to better visualize the bony labyrinth. The malformation may then be classified and additional treatment planned. For the very rare malformations where the inner ear (Michel aplasia) or cochlea (cochlear aplasia) is absent, a cochlear implant isn't a treatment option. These children should be considered for an auditory brainstem implant (ABI) for direct stimulation of the cochlear nucleus (Sennaroglu *et al.*, 2016). This may also be considered in case of common cavity or very severe hypoplasia, although a CI usually is considered a primary treatment option. The same argument may be used on auditory nerve aplasia. In a true nerve aplasia, a CI is obviously not a treatment alternative but an ABI is performed. However, cases with no sign of auditory nerve on MRI may still respond to electrical ABR (eABR) and electrical stimulation if implanted with a CI (Acker, 2001). This indicates that 3 Tesla MRI resolution is not sufficient to visualize the most pronounced nerve hypoplasia cases. For this reason, a CI, at least if preceded by positive eABR result, is possibly a better treatment option than an ABI, especially in view of the risks inherent in manipulating and stimulating the brainstem.

Although early surgery, today often between five-nine months of age, is the routine for pediatric cochlear implantation the finding of a malformation usually delays the intervention. This is often related to referral to a tertiary hospital. In the studies in this thesis, the average age of implantation was 1.8 years in the x-linked group (range=0.9-2.8 years, control group average age 1.9 years, range=0.7-5.5 years). In Sweden, the Karolinska University Hospital is responsible for the assessment, cochlear implantation procedure, and first year follow-up of all children with temporal bone malformations. An average of our material over the last five years reveals that 21% of the pediatric cochlear implant recipients have some kind of abnormality to the temporal bone.

Several authors have addressed the challenges related to cochlear implantation of a malformed cochlea (Papsin, 2005, Sennaroglu, 2010). The transmastoid-facial recess approach can be used in the large majority of malformation cases. When this is not possible it is usually related to a deviant route of the facial nerve. A standard approach was used in all 15 cases of cochlear implantation in the children with IP-3 in paper III and IV and no abnormalities of the facial nerve were encountered. As expected, a gusher occurred on opening of the cochlea in all cases. This is a very consistent finding in all reports on IP-3 surgeries. More often, a slow perilymph leak, oozing, may be found. This is seen in several conditions, such as IP-2 and some, but not

all, hypoplastic cochleae. The overall rate of gusher and oozing varies widely. Papsin (2005) reports 6.7% (n=103) CSF outflow and Sennaroğlu (2010) 30% (n=71).
With the opportunity to perform cochlear implantation in all children with a temporal bone malformation at Karolinska University Hospital follows a responsibility to report our results from this group of pediatric recipients. This is a unique opportunity to gather information from a national perspective and scrutinize each malformation subtype. By chance, Karolinska has seen unusually many children with *POU3F4* related inner ear malformation, IP-3, even compared to cochlear implant centers in larger countries.

COCHLEAR IMPLANT TREATMENT OF *POU3F4* DEAFNESS

In 1968, Olson and co-workers described a case of CSF leak and stapes fixation (Olson *et al.*, 1968). This was followed by a report in 1971 by Nance and colleagues describing a progressive mixed hearing loss in males (Nance *et al.*, 1971) where the conductive component of the hearing impairment was caused by stapes fixation. Attempting stapes surgery in these patients resulted in a perilymphatic gusher. The syndrome was described as Nance deafness or Perilymphatic gusher-deafness syndrome and was recognized to be inherited in an x-linked manner. In addition to the often rapidly progressive hearing loss, these patients exhibited a reduced vestibular response. Later, anatomical dissections discovered a wide internal auditory canal, and this was verified with polytomography (Cremers *et al.*, 1985) and CT (Phelps *et al.*, 1991). This also discovered the absence of bony cribriform plate between the internal auditory canal and the basal turn of the cochlea. As described earlier, de Kok and colleagues in 1995 identified the gene on the X-chromosome, *POU3F4*, responsible for the malformation.

With the experience of gusher and deterioration of hearing related to stapes intervention, hearing aids were the recommended therapy at the time. Using a BAHA has been considered but there is no attempt reported. Case reports of this condition, review male children with moderate-severe mixed hearing loss and attempts for stapes surgery resulting in a CSF gusher. An increase in sensorineural component (worsening bone conduction thresholds) is seen in the postoperative period. The children were fitted with conventional hearing aids, however with poor auditory outcome, and they usually entered deaf schools, at the best aiming for total communication (Carlson and Reeh 1993).

Cochlear implantation was regarded as contraindicated because of the severity of malformation of the modiolus and fundus of the IAC (Phelps, 1992). There were obvious concerns related to the management of the gusher and the possibility of postoperative rhinorrhea, as well as the risk of electrode displacement to the IAC. In 2006, Sennaroğlu and colleagues reported cochlear implantation in one child with x-linked deafness (Sennaroglu *et al.*, 2006). Describing this as a feasible method, and also adding the malformation to their classification system, it opened up CI treatment in this category of children. They described a 20-minute CSF gusher and insertion of a short straight electrode. Rhinorrhea occurred at day three requiring the placement of a lumbar drain for four days. In 2007 the first child with x-linked malformation received a cochlear implant at Karolinska. The boy was 1.6 years old and received a MED-EL Pulsar device to his right ear. A round window approach was used. A first insertion with a test electrode revealed IAC positioning on intraoperative x-ray but a straight Medium (24 mm) electrode was successfully placed within the cochlea, the position confirmed by conventional x-ray. At day two the boy experienced rhinorrhea, however gradually decreasing during six

days of conservative treatment and a lumbar drain was not used. During the following eight years ten children have received cochlear implants, five of them bilateral in a sequential procedure.

Other implant centers have reported cochlear implantation in children with x-linked deafness. In the years after Sennaroğlu's initial report (Sennaroglu *et al.*, 2006) four groups described their experience (Incesulu *et al.*, 2008, Aschendorff *et al.*, 2009, Lee *et al.*, 2009, Stankovic *et al.*, 2010). These groups describe difficulties during implantation, with IAC dislocation of the electrode array and different methods for CSF leak management. Incesulu *et al.* (2008) reported on four patients that were implanted with perimodiolar electrodes without lumbar drain. One of the patients required re-operation shortly after the initial procedure as the electrode had been inserted into the IAC. Two complications were reported, one patient developed facial nerve stimulation after five years requiring a replacement implant and a second developed a device failure after four years and received a new implant on the contralateral side. Aschendorff and colleagues (2009) describe an implantation in an adult case with radiological assistance during implantation to verify the array position of a perimodiolar electrode. Lee *et al*. (2009) reported on three patients implanted with straight arrays. All patients were successfully implanted and they describe an uneventful post-operative period and audiometric responses were obtained at loading four weeks after implantation. Intra-operative management was not discussed. Furthermore, Stankovic and co-workers (2010) reported four patients implanted and followed for a period of 2 to 6 years. They were implanted with straight arrays and had lumbar drains inserted before cochleostomy. The CSF gusher was controlled with placement of a lumbar drain lowered to 10-15cm below the external auditory meatus. In spite this, one patient required revision surgery for rhinorrhea at day seven and another at one year. In 2013 Kang and colleagues reported four children, two implanted with straight electrodes and two with perimodiolar. One case required revision surgery due to electrode insertion into the IAC and postoperative CSF leak.

During the last year an additional four groups have reported on cochlear implantation in children with x-linked malformation (Cosetti *et al.*, 2015, Choi *et al.*, 2016, Kim *et al.*, 2016, Saeed *et al.*, 2016). Cosetti *et al*. (2015) described five cases whereof two were revisions. They used a fluoroscopy technique with multiple exposures during implantation and also recommended an oval-shaped cochleostomy to better control the array during implantation. Choi and co-workers (2016) studied 11 subjects with POU3F4 verified mutation and implanted eight of them (three only presented with a moderate hearing loss). Their results indicated that auditory outcome is worse compared to age-matched controls implanted without inner ear malformation. No link was seen between type of mutation and initial auditory performance. In an attempt to compare mutation type (genotype) to postoperative outcome (phenotype) possibly those with a truncation or deletion performed worse. Kim and colleagues (2016) reported on bilateral sequential implantation on one child only without complications and Saeed *et al*. (2016) described bilateral implantation in two children. Postoperative CSF leak as rhinorrhea or fluctuant postauricular swelling complicated the procedure in all cases leading to a recommendation of obliteration of the Eustachian tube and middle ear in all cases.

The results of our procedures are reported in paper III, concluding that cochlear implantation in x-linked malformation cases is a safe procedure. The gusher should be waited out as it ceases

after approximately 15 minutes and attempts of electrode insertion prior to that is likely to increase the risk of IAC placement. The seal around the electrode at the opening to the cochlea has to be meticulously packed with circumferential soft tissue to avoid postoperative CSF leak, presenting as rhinorrhea. In our experience, there is no need for a lumbar drain. Only one case in paper III had postoperative rhinorrhea, starting at day two. Our management at the time was conservative. Although this was successful, the presently recommended management strategy for postoperative CSF leak in cases with x-linked malformations is a revision procedure and renewed packing with soft tissues. Sealing by additional surgical obliteration of the middle ear space or by a subtotal petrosectomy and blind end closure of the external auditory canal should be regarded as a salvage procedure only.

Of the 40 cases of primary pediatric cochlear implantation described in the studies above, a straight electrode was used in 25, uni- or bilaterally. There is no obvious correlation between the electrode type and the complication rate. However, theoretically, a precurved electrode could cause more damage if it were inserted in the IAC and then withdrawn, having curled around neural structures or the membranous labyrinth. It must be stressed that intraoperative imaging is mandatory, as there is a high risk of IAC displacement of the electrode, 20% (3/15) in our experience. Failure to recognize this will not only lead to absent post-operative speech understanding but also to a risk of facial nerve stimulation (Cosetti *et al.*, 2015). Simultaneous bilateral implantation is described in one child (Cosetti *et al.*, 2015), however, in the author's opinion this is not to recommend since the side in need for a revision surgery will not be known, in case a postoperative rhinorrhea develops.

In the eleven studies above, including paper III, cochlear implantation is described in two female children only, one in Incesulus report and one in paper III. Naturally, x-linked deafness is predominantly seen in males. Incesulu *et al.* (Incesulu *et al.*, 2008) questioned if true IP-3 malformations really can occur in females and attributed their origin to other reasons. However, genetic analysis was not performed in this study, and Marlin and co-workers (Marlin *et al.*, 2009) described eight females with verified *POU3F4* mutations, one of them with a typical IP-3. The same applies to the subject in paper III, with a large heterozygous *POU3F4* deletion and a typical IP-3 appearance of the malformation. Possibly this could be explained by skewed X-chromosome inactivation (Minks *et al.*, 2008). In 1998, Papadaki and colleagues (Papadaki *et al.*, 1998) reported on two sisters with severe mixed hearing loss and temporal bone imaging consistent with IP-3, although genetic testing was not performed. A moderate hearing loss could be seen in two of the seven female *POU3F4*-carriers with normal temporal bones in Marlin's study (Marlin *et al.*, 2009). The same type of hearing loss was seen in the three male subjects in Choi's study (Choi *et al.*, 2016), none of whom received a CI. This confirms that *POU3F4* mutations and IP-3 malformations do not necessarily lead to a severe-profound hearing loss even in male subjects. Apparently the phenotype varies, and as patients with a moderate hearing loss often neither is subject to genetic testing or temporal bone imaging, the true prevalence of x-linked malformation is unknown.

HEARING WITH A COCHLEAR IMPLANT AND X-LINKED MALFORMATION

The majority of the studies cited above focus on the surgical procedure and complications. Although most of them also report auditory outcomes, the follow-up time is often short and the performance measures are limited, only stating the occurrence of response to an auditory

stimulus or hearing thresholds with the CI. Lee *et al.* (Lee *et al.*, 2009) report good outcomes in two children, who achieved open-set word performance of 96 and 64%, and one child with minimal speech but improvement in sound detection (although mental retardation may have complicated assessment in this case). On the contrary, Stankovic (Stankovic *et al.*, 2010) discuss four patients (age at implantation between 1.1-3.7 years) where auditory perception post-implantation had not progressed past sound or single word detection. One patient achieved better speech perception but only due to contralateral amplification with a hearing aid. Cosetti (Cosetti *et al.*, 2015) describe three children with at least one year follow-up and suggests that good auditory performance may be achieved in x-linked malformation, since these patients were able to perform open-set multisyllabic word recognition. Kang and colleagues (Kang *et al.*, 2013) followed three patients for 1.5-6.4 years and reported on good auditory outcome, with no difference when comparing performance with age-matched patients with normal cochleae. This result was obtained by using scores from the Meaningful Auditory Integration Scale (MAIS) and Categories of Auditory Performance. However, these scales are based on estimations by parents or audiologists and not on objective testing. Finally, Choi (Choi *et al.*, 2016) reported on eight patients with a two year follow-up, finding a significant difference in CAP scores at two years compared with age-matched controls without inner ear malformation ($p<0.05$). Both paper III, comparing audiological outcome with a mean of pediatric recipients of a national two-center study and paper IV, comparing to a control group, shows significantly lower performance in speech perception, both in quiet and in noise, in spite of normal hearing thresholds with CI. No statistical difference was seen in sound localization. There seems to be some variability in outcome, but given the data of these previous studies and the papers in this thesis, in total, the concluding trend must be that children with x-linked malformation performs worse in functional auditory outcome although good hearing thresholds with a CI may be obtained. This indicates poorer sound processing in the x-linked group and may be linked to limited spectral and/or temporal resolution related to intracochlear issues, such as an abnormal spiral ganglion neuron organization. It may also be related to inferior nerve signal transmission capacity or an altered ability of central sound processing.

X-LINKED MALFORMATION, POSSIBLY A SYNDROMIC HEARING LOSS

Today, *POU3F4* related x-linked malformation is regarded as a non-syndromic hearing loss. In the studies above only a few authors mention symptoms or findings other than hearing loss, such as the children's behavior, on assessment and follow-up.

It has previously been reported that children with x-linked deafness exhibit features including testing difficulties. Stankovic suspected cognitive and developmental delays associated to the hearing loss. They go on to suggest that the developmental delay and loss of hearing could be associated through the malfunctioning of *POU3F4*. Choi, on the other hand, stated that no syndromic feature was seen during the two year follow-up. Reviewing the literature on x-linked malformation or *POU3F4*, there are occasional reports describing behavioral features of these patients. (Carlson *et al.*, 1993) reviewed three children with x-linked deafness and hearing aids. On describing the children they state that "*KM continues to demonstrate severe developmental delays in speech-language, visual-motor integration, and fine motor skills. These difficulties contribute to his persistent insecurity and apprehension in the test situation.*". Regarding his half-brother, the same authors summarize "*MB was diagnosed as having attention deficit*

disorder in 1988 and subsequently placed on Ritalin for his hyperactivity. He continues to be followed in the child development clinic for his behavioral and speech/language difficulties.".

Our clinical experience during a relatively long follow-up revealed a group of children with complex needs. In paper IV older children (>4 years, n=6) with POU3F4 verified x-linked malformation hearing loss are examined, and the results indicate that they exhibit difficulties not only in the domains of hearing but also language, and some subdomains of cognition and mental health, compared with a hearing-matched group. These findings correlate well with reports, as with the example above, stating neurodevelopmental difficulties. Four of the older boys in paper IV received an ADHD diagnosis. The cognitive performance of the x-linked group could be related to alterations in central function during embryogenesis. Controlling early development, linked to the development of the neural tube, in mice the gene *pou3f4* has a regulatory role which is not fully clarified. As it is expressed in periotic bone during labyrinthine development as well as brain structures, including frontal lobes, it is likely to have a role in early stages of embryogenesis in both areas. *Pou3f4* mutations are known from animal studies to lead to inner ear malformations (Phippard *et al.*, 1998, Minowa *et al.*, 1999), however, to the author's knowledge, there are no behavioral studies on mice to support a neurodevelopmental disorder.

To conclude, based on our present knowledge of *POU3F4* in human and animal studies, previous indications of altered behavior, cases of confirmed ADHD and the findings in paper IV it seems unlikely that the hearing loss alone explains the features demonstrated by paper IV. X-linked hearing loss is today classified as a non-syndromic genetic hearing loss related to mutations in the *POU3F4* gene or surrounding domain. However, the correlation between type of malformation, similar genetic findings and consistent behavior in our study group indicates that this type of hearing loss may be part of a syndrome including hearing loss and neurodevelopmental disorder consistent with hyperactivity and attention deficit.

FUTURE PERSPECTIVES

The cochlear implant is the worlds most developed bionic device and has opened the field of electrical intervention. The knowledge obtained from this system of electrical stimulation will be useful in other areas. The concept of sensory electrical stimulation is today expanded to vestibular or ocular implants, currently in clinical trials. Other areas using electrical stimulation include deep brain stimulation. Although there are attempts at using infrared laser light for neural activation (Fridberger *et al.*, 2006, Tan *et al.*, 2015), electricity is likely to continue to be the main mediator due to its relative simplicity and the substantial clinical experience that has been accumulated. Efforts should continue in the direction of improving the neural interface, in a cochlear implant the intracochlear electrode array. Several attempts are currently made for improving the properties of the electrode, for instance by including slow release mechanisms for intracochlear medication and less traumatic electrode properties. Both are import for strategies for improving hearing preservation. The concept of minimizing trauma during surgery is also important for ease of future revision surgery and optimizing the possibilities of alternative treatments.

The use of cochlear implants has expanded to new patient categories. It is no longer a device for profoundly deaf patients only. The indications have evolved over the last decades including

the domains of this thesis, patients with residual hearing and children with malformed cochleae. Both groups of patients were previously judged as unsuitable candidates but efforts by a large number of research groups have shown that cochlear implantation not only is possible but also an intervention with good outcome.

Today, it is possible to preserve residual hearing in most patients receiving cochlear implants. One of the most important remaining questions is how to maintain results. Further work is necessary to understand the mechanisms that are involved in cases of late loss of hearing after implantation, as well as possible direct treatments. The area of drug delivery to the inner ear has only started.

With increasingly better resolution on CT and MRI, the work on classifications of the malformations must continue. In addition, for the unusual malformation IP-3, histopathological studies will be essential to understand the organization of the membranous labyrinth and SGN. This may reveal precise explanations to the conductive component of the hearing loss as well as to the poorer audiological outcome on electrical stimulation. For all malformations genetic analysis is likely to be increasingly important. In cases of malformations, bony as well as membranous, it is necessary to clarify the regulatory roles of the genes involved in embryogenesis. Today these are not sufficiently known to explain the diversity of phenotypes expressed. Combining the domains of imaging, histopathology and genetics will be the most successful way forward in understanding the mechanisms involved in the subtypes of temporal bone malformation. There is also a need for increased knowledge in the complex system of auditory pathways, from the cochlea to a subcortical and cortical level, to comprehend how we learn to hear.

Evaluating the malformation subtype IP-3 as in this thesis is one step forward. Every category of malformations should be analyzed, identifying the specific difficulties for that group. A difficulty in this lies in the small number of children examined at each center, and that the possibility of long term follow-up may be limited. To centralize the malformations of a country to one center, as in Sweden, is a first step but future work should include international collaboration. As a first step for this to be successful a consensus of comparable assessment tools is necessary.

The over-all aim of the study in paper IV was to embrace a “total evaluation” of medical, psychological and social parameters for a comprehensive approach to the child with a cochlear implant. This aim, to draw the “complete picture”, is important for analysis of how to best assist in training the child and counsel and support the family on an individual basis. For future studies of groups of pediatric cochlear implantation recipients, this concept is likely to become increasingly important. “Good outcome” is not only about a successful surgical procedure, or development of speech and oral language, or socio-psychological well-being of the patient, but rather optimizing each area for a comprehensive good result. Understanding of, and coping with, the unique difficulties each individual child faces growing up with a cochlear implant is a key to a successful treatment.

CONCLUSIONS

Paper I. The guinea pig cochlea exhibits high resilience provided the use of a surgical technique with limited trauma. Cochleostomy and implantation itself does not alone explain postoperative permanent loss of residual hearing when occuring. Very limited to no signs of histological changes in hair cells and spiral gangloin neurons are seen after short follow-up.

Paper II. Electrocochleography and micro-CT shows that endolymphatic hydrops is present during the first week after cochlear implantation. The soft tissue response does not increase at longer follow-up.

Paper III. Cochlear implantation is a feasible and safe alternative for hearing restoration in children with x-linked malformation. Complications are few and can be managed during the surgical procedure. With a cochlear implant the children attain hearing but at a lower level compared to average CI recipients and they develop spoken language.

Paper IV. Children with x-linked malformation exhibit, in addition to their severe-profound mixed hearing loss, features of a neuro-developmental disorder in the area of attention deficit and hyperactivity. Their cognitive abiliteis are below those of a control group of pediatric CI recipients. These findings does not seem related to their hearing loss alone but instead to their *POU3F4* mutation. This concludes a suggestion to re-classify *POU3F4* related x-linked deafness as a syndromal hearing loss.

SAMMANFATTNING PÅ SVENSKA

Hörselnedsättning är ett av de vanligaste handikappen i världen. Hörapparater är ofta en bra hjälp men de fungerar inte för patienter med grav hörselnedsättning eller dövhet. Möjligheten att använda cochleaimplantat (CI, hörselimplantat i snäckan) hos dessa patienter är ett stort medicinskt framsteg som revolutionerat behandlingen av svåra hörselhandikapp. Fler än fyra tusen vuxna och barn har opererats i Sverige under de senaste decennierna, i världen har flera hundra tusen opererats.

Vår hörsel bygger på att ljudet som leds till innerörat kan omvandlas till nervsignaler av snäckans (cochleans) sinnesceller. De kallas hårceller och om de inte fungerar kan ljudet inte fortledas till hjärnan. Vanliga hörapparater, som förstärker ljud, kan hjälpa människor med lätta till måttliga hörselnedsättningar men vid uttalade besvär minskar vinsten av att förstärka ljudet, eftersom snäckan då saknar förmåga att skapa nervimpulser. CI innebär att man blir oberoende av snäckans funktion och istället stimulerar hörselnerven direkt. Under de senaste femtio åren har metoden för CI utvecklats successivt. I början handlade det om enkla försök med att stimulera hörselnerven elektriskt och på 1970-talet opererades de första patienterna. Dagens system är tekniskt mycket avancerade men grundprincipen är densamma, att elektriskt stimulera hörselnerven och på så sätt alstra nervimpulser som av hjärnan uppfattas som ett hörselintryck. De patienter som kan bli hjälpta med CI är dels dövfödda barn som opereras tidigt under barnaåren och på så sätt kan lära sig att förstå hörselintryck och därmed utveckla tal, dels barn eller vuxna som blivit döva och återfår hörselförmågan med CI. Tekniken i cochleaimplantaten utvecklas kontinuerligt för att ge bättre talförståelse, bättre möjligheter att uppskatta musik och vara lättare att använda. Trots att cochleaimplantat fungerar bra för flertalet användare finns det fortfarande områden där vi behöver lära oss mer. Den här avhandlingen handlar om två patientgrupper med särskilda problem. Dels de med ”partiell dövhet”, dels de som föds med inneröremissbildningar.

Partiell dövhet

Bland vuxna som opereras idag finns en ökande grupp med varierande grad av kvarvarande hörsel i basregistret (”residual hearing”). Denna hörselrest är som regel liten och patienten är diskantdöv med en kraftigt begränsad hörselfunktion, trots maximalt stöd av hörapparater. I samband med en rutinmässig CI-operation är risken stor att patienten helt eller delvis förlorar den kvarvarande hörseln. Orsaken till detta är oklar. Om man kan bevara patientens egen, naturliga, bashörsel och kombinera denna med elektrisk hörselstimulering via CI i diskanten i samma öra får patienten sammantaget ett bättre hörselresultat, och det blir till exempel lättare att höra i bullriga miljöer. En mindre traumatisk kirurgisk teknik har utvecklats (hörselbevarande CI-kirurgi) och denna kan kombineras med en kortare och mjukare implantationselektrod. Trots detta förlorar ungefär 25 % av patienterna sin kvarvarande bashörsel i samband med kirurgin. Avhandlingens två första delarbeten är djurexperimentella och handlar om att öka förståelsen av vilka sjukdomsmekanismer som bidrar till att den kvarvarande hörseln skadas i samband med kirurgi.

I **studie 1** bedöms hur hörseln och snäckans inre strukturer påverkas av olika grader av kirurgiskt trauma. Tre grupper marsvin opererades med: i grupp 1 endast cochleostomi (öppnandet av ett hål till snäckan), i grupp 2 utfördes en djup cochleaimplantation och i grupp

3 en grund cochleaimplantation. Hörseln testades med hjälp av hjärnstamsaudiometri 7, 14 respektive 28 dagar efter operationen. Resultaten visar att marsvinets hörsel till stor del kan bevaras även om man borrar upp ett hål i snäckans nedersta vindling (grupp 1) liksom om man för in en elektrod en kort sträcka in i snäckan (grupp 3). Om man däremot för in den djupare förloras all hörsel (grupp 2). Försöksdjuren var normalhörande och hörseln kunde testas även på de höga frekvenser som låg i närheten av implantatelektroden. Mikroskopisk undersökning av snäckan på de marsvin som varit implanterade med en kort elektrod visade efter avslutad uppföljningstid, 28 dagar, att snäckans inre strukturer till stor del bevarats, hårcellernas antal var nära oförändrat i 3 av 5 snäckor och att nervcellernas antal var oförändrat jämfört med icke-implanterade snäckor. Slutsatsen är att marsvinets snäcka har hög motståndskraft för måttligt kirurgiskt trauma och att cochleostomi eller kort implantation inte ensamt förklarar hörselförlust, i de fall det inträffar.

I **studie 2** undersöks hypotesen att cochleaimplantation ger så kallad endolymfatisk hydrops. Det innebär att man har ett övertryck i den ena av innerörats vätskor, endolymfan. Vid mikroskopisk undersökning av snäckor som varit cochleaimplanterade har man, hos såväl marsvin som människa, noterat endolymfatisk hydrops i ungefär 25 % av fallen. Hydrops, övertryck, har även kopplas till Menières sjukdom som ger fluktuerande yrsel, tinnitus och hörselnedsättning. I studien cochleaimplanterades fyra grupper av marsvin med en kort implantatelektrod, och djuren följdes sedan i upp till 72 dagar. Elektrocochleografi (mätning av elektriska svar i snäckan vid ljudstimulering) samt mikro-DT (röntgen med datortomografi) användes för att undersöka förekomsten av hydrops. Studien visar att det finns tecken till endolymfatisk hydrops under första veckan efter cochleaimplantation.

Barn med missbildade inneröron

Ungefär 20 % av de barn som opereras med CI vid Karolinska Universitetssjukhuset har ett missbildat inneröra. Andelen är relativt hög då Karolinska har rikssjukvård på denna patientkategori. Missbildningar av snäckan klassificeras övergripande i Incomplete Partition (ofullständig uppdelning av snäckans inre strukturer), Hypoplasia (liten snäcka) och Common cavity, där innerörats hörseldel (snäckan) och balansdel är sammansmält i en struktur. En undergrupp till Incomplete partition (IP) är typ 3 (IP3) som har en snäcka med kraftigt förändrad inre anatomi, där de beniga delarna i snäckans centrum och golv saknas vilket innebär att mjukdelarna, själva hörselorganet med hårcellerna (Cortiska organet) och nervstrukturerna (nervcellerna och hörselnerven) saknar sitt naturliga skelett. Dessutom innebär detta en stor öppning mot inre hörselgången, som i sig är vidare än vanligt, med en öppen kommunikation till den cerebrospinalvätska som omger hjärnan. Denna missbildning är kopplad till mutationer i en gen (*POU3F4*) på x-kromosomen. Den kallas därför ofta ”x-linked malformation” (x-bunden missbildning). Diagnosen sätts genom fyndet av en grav hörselnedsättning och den typiska inneröremissbildningen, som visualiseras med hjälp av datortomografi och magnetresonanskamera. Genetisk testning verifierar diagnosen. X-bunden missbildning klassificeras idag som en icke-syndromal hörselnedsättning, d.v.s. att den inte ger några andra symtom än hörselnedsättning. Avhandlingens två sista studier undersöker möjligheterna till, och effekterna av, cochleaimplantatbehandling på 10 barn med x-bunden hörselnedsättning och inneröremissbildning.

I **studie 3** utvärderas den kirurgiska metoden, dess risker och barnens språkutveckling. Studien visar att cochleaimplantation kan genomföras säkert, utan risk för allvarliga komplikationer, men att implantatelektrodens läge måste verifieras under ingreppet för att säkerställa att den

inte lagt sig i inre hörselgången. Med cochleaimplantat utvecklar barnen hörsel, men på en nivå som är lägre än genomsnittet för CI-barn utan inneröremissbildning. De lär sig talspråk och kan gå i vanlig skola men behöver ofta extra stöd i undervisningen.

Studie 4 tar fasta på en klinisk erfarenhet att dessa barn, utöver hörselhandikappet, har uppmärksamhetssvårigheter och hyperaktivitet. Hypotesen är att mutationer i genen *POU3F4* på x-kromosomen inte bara ger inneröremissbildning med hörselnedsättning, såsom det antas idag, utan också ett neuropsykiatriskt funktionshinder. I denna studie jämförs de med en kontrollgrupp barn som har CI av annan anledning (10 barn med *Connexin26* mutation). Resultaten av genetisk analys, fördjupad språkutredning, psykologbedömning och undersökning av barnens psykosociala hälsa visar att barnen har svårigheter med impuls- och aktivitetskontroll, uppmärksamhet och socialt samspel som inte bara kan förklaras av de kommunikationssvårigheter som hörselnedsättningen ger. Den samlade bilden liknar en ADHD-problematik, och några av de äldre barnen har fått den diagnosen verifierad. Vi föreslår därför att x-bunden missbildning (*POU3F4* mutation) omklassificeras till en syndromal hörselnedsättning.

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