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Hormonal effect on the inner ear: Two endocrine syndromes

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Hormonal effect on the inner ear: Two endocrine syndromes

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Till mamma och pappa

ABSTRACT

Hearing loss is a major problem in our society with more than 5 % of the population world-wide suffering from disabling hearing loss according to the WHO. There are many endocrine syndromes associated with hearing loss. In both Turner syndrome and Pendred syndrome hearing loss is a prominent feature often leading to a need for hearing rehabilitation with hearing aids or cochlear implants. Hormonal treatment might also affect the inner ear directly and studies have shown a negative effect on hearing by progesterone treatment.

The overall aim of this thesis is to enlighten the close connection between hearing and endocrinology both directly via hormonal effects on the inner ear and indirectly by which endocrine syndromes have an impact on both hearing and endocrine target organs.

Studies I and II explore the audiological features in young women with Turner syndrome and the effect of hormonal treatment. Sensorineural hearing loss is common with high-frequency hearing loss and mid-frequency dip being the configurations most often seen. The mild to severe hearing loss concentrated to the high- and mid-frequencies leads to reduced speech perception which leads to the necessity of hearing rehabilitation with hearing aids. No effect on hearing by the hormone replacement therapy with estrogen was seen but a negative effect on high-frequency hearing, attributed to the growth hormone treatment, was found.

Study III investigates the presence of progesterone receptors in the inner ear which could explain a direct negative effect on hearing by progesterone containing hormone replacement therapy. No nuclear progesterone receptors were found according to the results of the immunohistochemistry, in rat and human, and by PCR and Western blot in rat. Therefore, direct nuclear effect seems unlikely.

Study IV investigates the natural hearing history and outcome after cochlear implantation in children with severe hearing loss due to Pendred syndrome or DFNB4 having LVAS and/or IP2-malformation. A severe to profound hearing loss is seen, often with an early onset, but progressive hearing loss is not rare. The speech and language tests show low results in expressive vocabulary and pragmatic skills but normal results in receptive vocabulary. Additional symptoms are vertigo, fluctuating hearing, motor problems, concentration deficits and sleeping disturbances.

In conclusion, hearing impairment is common and linked to several endocrine syndromes including Turner syndrome and Pendred syndrome. In both syndromes the hormonal and genetic impact can lead to severe hearing impairment though the risk for congenital severe hearing loss is present only in Pendred syndrome. An early rehabilitation with hearing aids or cochlear implants in regard to the hearing deficit is necessary to prevent social isolation and to ensure speech development and speech perception. Hormonal treatment might interfere with hearing but no direct nuclear effect of progesterone on the inner ear was found.

LIST OF SCIENTIFIC PAPERS

This thesis is based on the following studies, which will be referred to in the text by their roman numerals:

I. *Audiometric features in young adults with Turner syndrome.* Bonnard Å, Hederstierna C, Bark R, Hultcrantz M.Int J Audiol. 2017 Apr 19:1-7. doi: 10.1080/14992027.2017.1314559.

II. *The effect of hormonal treatment on hearing in young women with Turner syndrome: A cohort study.* Bonnard, Å., Hederstierna, C., Bark, R., Hultcrantz, M. Manuscript

III. *No direct nuclear effect of progesterone in the inner ear: other possible pathways.* Bonnard, Å., Sahlin, L., Hultcrantz, M., Simonoska, R. Acta Oto-Laryngologica, (2013) 133:12, 1250-1257, DOI: 10.3109/00016489.2013.825377

IV. *Social skills, hearing and language outcome after cochlear implantation in relation to cochlear malformation in children with LVAS and IP2 malformation.* Bonnard, Å., Löfqvist, U., Anmyr, L., Smeds, H., Wales, J., Karltorp, E. Manuscript.

CONTENTS

LIST OF ABBREVIATIONS

BDNF	Brain-Derived Neurotrophic Factor
BNT	Boston Naming Test
BRIEF	Behavior Rating Inventory for Executive Functions
CCC-2	A pragmatic skills questionnaire, Childrens Communication Checklist-2
CT	Computer Tomography
dB	Decibel
DFNB4	DeaFNess, Autosomal recessive 4
DPOAE	Distortion Product OtoAucoustic Emissions
HINT	Hearing In Noise Test
IGF-1	Insulin-like growth factor 1
IP2	Incomplete partition type 2
LVAS	Large Vestibular Aqueduct Syndrome
MRI	Magnetic Resonance Imaging
PCR	Polymerase Chain Reaction
PPVT	Peabody Picture Vocabulary Test
PR	Progesterone Receptor AB
PRA	Progesterone Receptor A
PRB	Progesterone Receptor B
PTA4	Pure Tone Average 4
SIR2	Speech and Intelligibility Rating scale 2

PREFACE

If you had a slight hearing loss, between 26-40 dB, you would have trouble hearing soft speech from a distance or speech in a background noise. If you had a moderate hearing loss, between 41-60 dB, hearing regular speech, even at a close distance, would be problematic. With a severe hearing loss between 61-80 dB, you may only hear very loud speech and surrounding noise, as loud as a fire siren or a door slam. Most conversational speech would not be heard. With a profound hearing loss, above 80 dB, you may only perceive loud sounds and vibrations.

(Adapted from WHO's grading of hearing loss).

Communication is important for all animals, including humans. The main communication mode, speech, is dependent on our ability to hear. We use our hearing continuously and for a wide variety of purposes. It is the sound of our parents' voices that calms us as babies in destress, the hearing that is necessary for listening to bedtime stories, exchanging thoughts, feelings and ideas with friends and colleagues as well as alerting us of danger, like detecting the sound of falling branches from a tree or the fire alarm. Most people in the world are using spoken language for communication but about 70 million deaf people worldwide have sign language as their first language (https://wfdeaf.org/faq/, 170610).

Hearing impairment is a major problem in our society and according to WHO (http://www.who.int/mediacentre/factsheets/fs300/en/ 170622) over 5% of the population in the world suffer from a disabling hearing loss. In Sweden, 6.0 % of men and 2.9 % of women between the ages of 18-50 years have a hearing loss, these percentages do not include the elderly with their higher incidence of hearing loss (1).

BACKGROUND

Anatomy and physiology of the ear

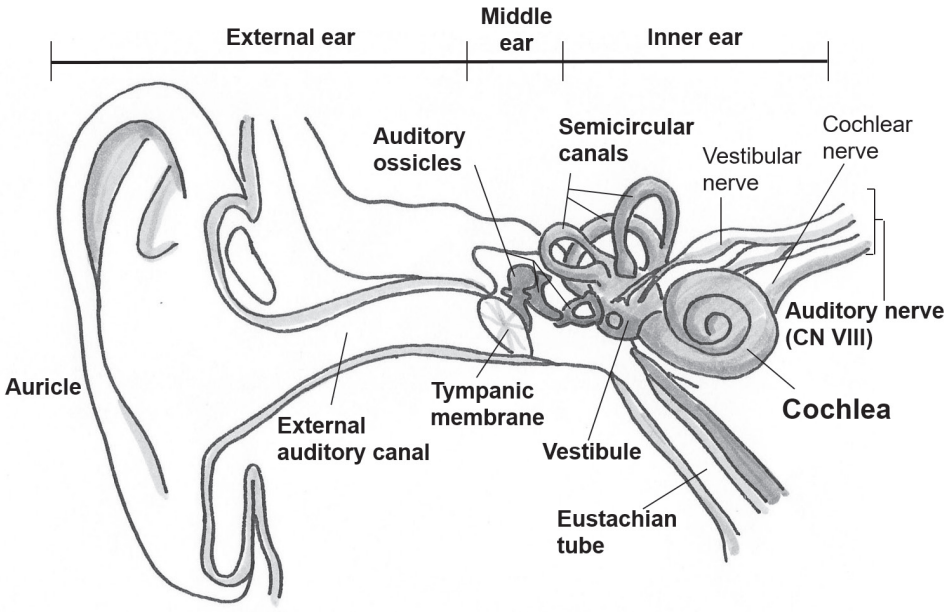

Figure 1: Schematic image of the human ear divided in external, middle and inner parts. Republished with the kind permission of Rusana Bark.

The ear transforms airborne soundwaves into electrical signals in three different steps. The soundwaves in the air are captured by the auricle (a part of the external ear) and continues through the auditory canal to the eardrum, the tympanic membrane. The tympanic membrane and the three auditory ossicles, the malleus, incus and stapes, compose the middle ear. The function of the middle ear is to amplify the sound by about 60 dB. By vibrating the tympanic membrane and the three middle ear bones the soundwaves from the air are transformed into a mechanical force further transmitted via the stapes footplate in the oval window to form pressure-waves in the fluid in the inner ear, the cochlea (figure 1).

The cochlea is a snailshell-like structure and normally makes two and a half turns in humans. It is divided into three channels: the Scala vestibuli, Scala media and Scala tympani, divided by the Reissners' membrane and the basal membrane. The pressure wave transmitted by the stapes footplate will travel inside the Scala vestibuli from the base of the cochlea to the apex and then back again trough the Scala tympani. The exceeding force will be eliminated

through the round window. The organ of Corti is located in the Scala media, which is situated between the Scala vestibule and tympani. This is the sensory organ of hearing where the pressure wave is transformed into an electrical signal. The organ of Corti contains one row of inner hair cells and three rows of outer hair cells covered by the tectorial membrane, where the stereocilia are embedded. When the pressure wave in the Scala vestibuli moves the basilar membrane, the hairs, stereocilia, on top of the hair cells will bend activating a cascade of events leading to the activation of the cochlear nerve (figure 2).

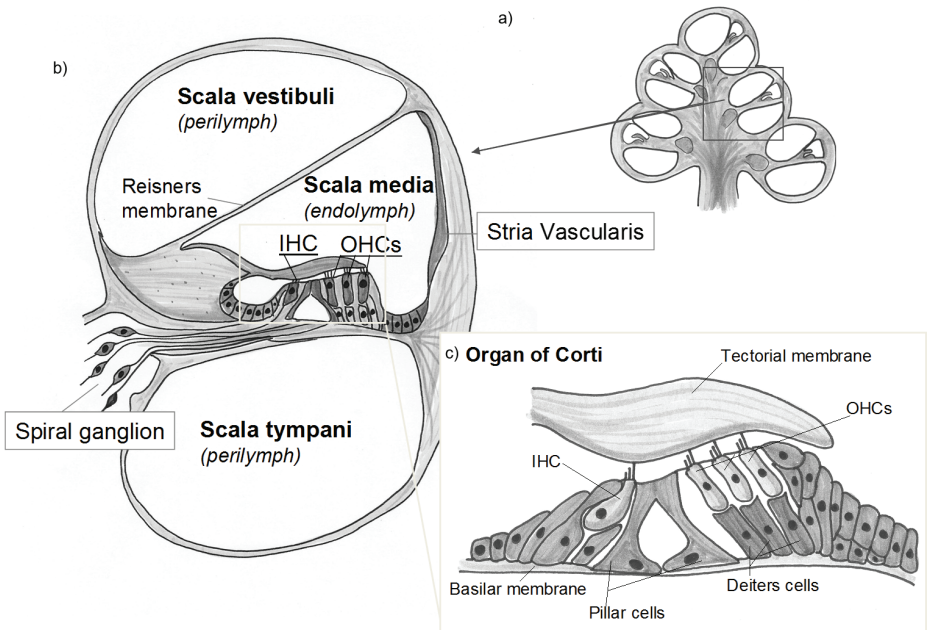

Figure 2: A drawing of the human inner ear anatomy with a mid-modiolar section (a) and an enlarged spiral canal with the three Scala, vestibuli, media and tympani and the spiral ganglion (b). The sensory organ of hearing, the Organ of Corti, is situated in the Scala media with its inner and outer hair cells (IHC and OHC) (c). Republished with the kind permission of Rusana Bark.

Depending on the frequency of the pressure wave, a specific area along the membrane will have a maximal reaction to the stimuli, i.e. the basilar membrane is tonotopic (2). The high frequencies will cause a maximal reaction in the basal turn of the cochlea and the low frequencies in the apex. In order for the hair cell to be able to release this electrical impulse, the ionic content of the surrounding liquids is essential. In the Scala media the endolymph is rich in potassium and the perilymph-containing Scala vestibule and tympani are rich in sodium creating an electrochemical gradient. When the stereocilia

deflect, mechanically gated ion-channels open and positively charged ions (potassium and calcium) flow into the cell from the surrounding endolymph and a depolarization follows. This opens voltage-gated calcium channels and the entry of calcium ions triggers the release of neurotransmitters that will activate the nerve. The positive ions are then transferred to the perilymph by the electrochemical gradient.

The spiral ganglion nerve threads, situated in the modiolus (middle) of the cochlea, comprise the cochlear nerve which connects to the cochlear nuclei in the brainstem. Nerve fibers continue through the brainstem, mid-brain and thalamus to the primary auditory cortex in the temporal region. The afferent system crosses over in the brainstem thus making the auditory input from one ear stimulate the cortex-region on both sides of the brain (figure 3).

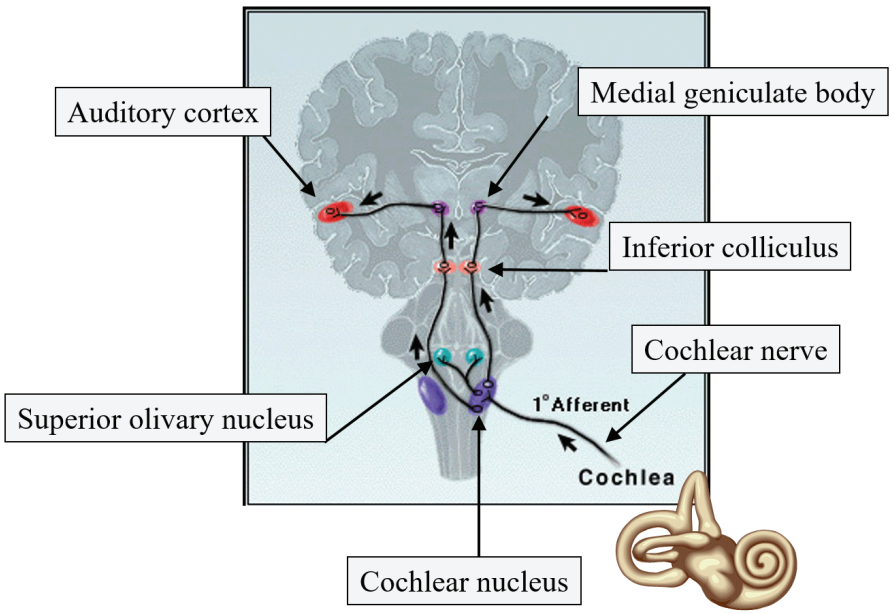

Figure 3: The central auditory pathways from the cochlea, via the cochlear nerve, the brainstem (cochlear nucleus), the pons (superior olivary nucleus), the mid-brain (inferior colliculus), the thalamus (medial geniculate body) and to the auditory cortex in the temporal lobe. The figure is showing the bilateral stimulation of the auditory cortex by the cross-over at the brainstem level. Republished with the kind permission of Rusana Bark.

Inner ear development and malformation

The inner ear is developed from the otic placodes that begin to be visualized at day 22 on the outside of the embryo. At the fourth embryonal week it has invaginated and formed an otic cyst that will become the future cochlea and vestibular organ. At 8 weeks the cochlea has its final 2.5 turns and during week 9-12 the organ of Corti is fully developed. Parallel to this, the vestibulo-cochlear nerve develops and a connection between the cochlea and the brainstem is established at 9 weeks. During the second trimester, the organ of Corti matures and auditory connections between the brainstem and the brain begin to develop (3, 4). Already by the end of the second trimester, around week 22, the fetus starts to hear. Interruptions in these processes lead to different malformation depending on the stage of development and genesis. Sennaroglu et al presented a new classification for vestibulo-cochlear malformations in 2002 based on the findings from computer tomography and magnetic resonance imaging in relation to the embryonic development (4). They divided the malformations into different groups depending on the time of developmental arrest. These malformations ranged from a complete lack of cochlea and vestibular organs to an almost normal cochlea with only an incomplete partition of the last turn, named an incomplete partition type 2 (IP2).

Incomplete Partition type 2 and Large Vestibular Aqueduct Syndrome
In IP2-malformation the inner ear is normal or slightly hypoplastic with a fused apex resulting in only 1.5 turns of the cochlea. This represents a developmental arrest in the seventh fetal week. There are different degrees of modiolar dysplasia and a histological study showed a reduced number of cells in the spiral ganglion with extensive inter-individual variation which might affect the hearing (5). A combination of a large vestibular aqueduct (Large Vestibular Aqueduct Syndrome, LVAS) and IP2 is common but both IP2 and LVAS can occur in isolation. These malformations can be seen in several syndromes such as Pendred, branchio-oto-renal, CHARGE and DiGeorge's syndromes as well as in a non-syndromic deafness (DNFB4). This autosomal recessive inherited malformation has in approximately 50% of the cases homo- or heterozygote mutations in the SLC26A4 gene, however a combination including a mutation in FOXI1 or KCNJ10 has also been found (6-8).

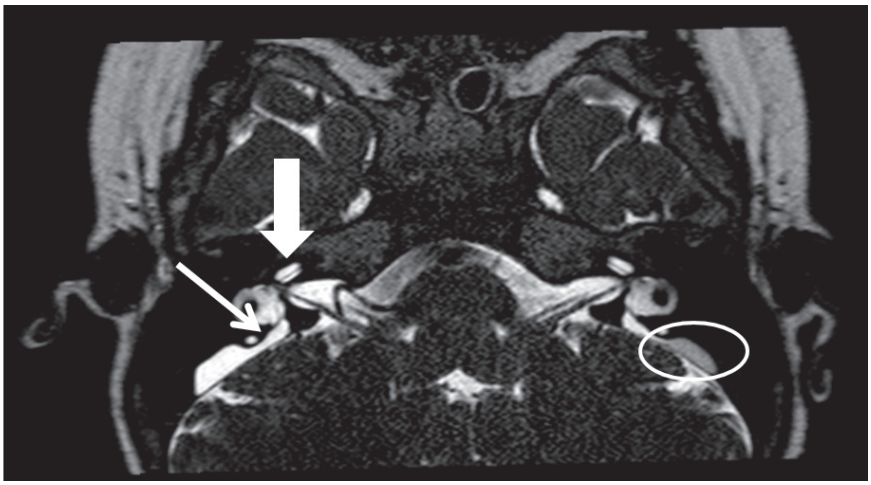

Figure 4: MRI of the skull base and the inner ears showing scalar asymmetry (thick white arrow), large vestibular aqueduct, LVAS (thin white arrow) and enlarged endolymphatic sac (circle).

Endocrine interaction with the auditory system

There are many endocrine disorders associated with hearing loss. Among these are a high frequency sensorineural hearing loss linked to IGF-1 deficiency, an increased frequency of sensorineural hearing loss in acromegaly, hypoparathyroidism and sensorineural deafness in Barakat syndrome and congenital hypothyroidism (endemic cretinism) composed of dwarfism and sensorineural hearing loss (9). The mechanisms differ from genetic mutations interfering with the fetal development resulting in inner ear malformation to ongoing processes due to hormonal insufficiency leading to an early hearing loss.

Turner syndrome

Turner syndrome is the most common sex-chromosome disorder in women and occurs in one out of 2500 live-born girls (10). Girls with Turner syndrome are born with a total or partial loss of one female sex-chromosome which leads to an estrogen deficiency due to a lack of or underdeveloped ovaries. Short stature (around 142 cm in non-treated), no spontaneous development of secondary female phenotypes and infertility are the main characteristics of the syndrome (11). There are several karyotypes where 1) 45,X have a total loss of one X-chromosome, 2) 46,X,i(Xq) have an isochromosomy of the q-arm, as well as 3) a mosaic of normal and Turner-type cells are the three most common ones. In the literature, having 45,X or 46,X,i(Xq) karyotype is

associated with a higher incidence of comorbidities such as congenital heart defects, hypertension, osteoporosis, auto-immune diseases, for example diabetes mellitus and hypothyroidism, as well as hearing disturbances (10, 12).

Ear and hearing problems are frequent in women with Turner syndrome and have a negative effect on their wellbeing and quality of life (13, 14). Throughout life, different periods of ear and hearing problems can be seen in girls and women with Turner syndrome. During childhood, there is a high incidence of otitis media resulting in a conductive hearing loss, frequent tympanostomy tube insertions and antibiotic treatment (15-17). Women with Turner syndrome also develop a higher incidence of chronic otitis media and cholesteatoma over time (18, 19). Sensorineural hearing loss in the form of a mid-frequency dip can be present as early as age five and progresses over the years (20, 21). A high frequency sensorineural hearing loss that develops in early adulthood is often present and this can have a devastating effect on the hearing situation for the individual. In such cases, hearing aids are often required earlier than in the normal population (12, 22-24). There is an increased risk for ear and hearing problems in women with karyotype 45,X or 46, X,i(Xq) (12, 16, 25).

Estrogen treatment is used in order to prevent osteoporosis and cardiovascular diseases as well as promote puberty. About one third of girls with Turner syndrome will start a normal pubertal development but less than 5 % will have a normal menstruation cycle. Fertility and estrogen treatment is begun around the age of 12 (11). The estrogen treatment is induced at low doses with pure estrogen and changed for a combination pill including a progesterone content depending on the stage of pubertal development. The treatment is ended by the time of menopause.

Historically, anabolic steroids have been the treatment of choice for height improvement, but in 1980 the first recombinant growth hormone treatment was introduced in Sweden. Today this is the treatment of choice and has been shown to be effective with few side-effects according to several studies (20, 26-30). In the first studies on growth hormone substitution an increased incidence of otitis media was seen, but this could not be confirmed in later studies (20, 27). There is however ongoing research on the effect of growth hormone and its metabolite IGF-1 on the inner ear, where IGF-1 is shown to be crucial for embryonic inner ear development. Girls with a low concentration of IGF-1 in childhood have a higher risk of sensorineural hearing loss (25).

Pendred syndrome

Pendred syndrome is a genetic disorder composed of a triad of symptoms, hearing loss, vertigo, and thyroid enlargement (goiter) (31). It has a mutation in the PDS-gene (SLC26A4) responsible for the Pendrin protein involved in bicarbonate (HCO^{3-}) transport in the inner ear and kidneys and iodine (I^-) transport in the thyroid gland (6, 32-34). In Pendred syndrome there is usually a homozygote mutation with one mutated gene in the genome from both parents. A heterozygote mutation (a mutation from only one parent) is more frequently accompanied with a non-syndromic hearing loss, DFNB4, where the thyroid is not affected. The KCNJ10 gene, known to be affected in some patients, is also responsible for an ion-receptor necessary for hair cell depolarization (8). FOXI1, a transcriptor-regulating gene, is another known gene involved in Pendred syndrome (35). This gene is necessary for the transcription of SLC26A4. As mentioned earlier, the specific inner ear malformation, IP-2 and LVAS, is seen in individuals with Pendred syndrome (36).

The hearing impairment problem in Pendred syndrome is of sensorineural type and ranges from mild to profound (31, 37). A child can be born with a profound hearing loss but it can also be progressive and fluctuation in hearing is common. In children with fluctuating hearing loss the adjustment of hearing aids poses a great problem as does speech and language acquisition due to a non-predictable hearing situation. A fluctuation in hearing ability can also be a reason for late identification of hearing loss due to variability in results on hearing tests. Head trauma or upper airway infections can provoke a worsening in hearing. Vertigo is present in about one third of the patients and is often displayed by episodes of vertigo ranging from seconds up to days (31, 37, 38). For some individuals, it can be provoked by a raised internal pressure in the inner ear as when doing sit-ups or the Valsalva maneuver. The thyroid enlargement manifests during the second decade of life and is usually euthyroid. Occasionally, thyroid hormone substitution is necessary (31, 38).

Hormonal treatment

The girls and women with Turner syndrome, as well as a significant number of women world-wide, are treated with combination pills containing estrogen and progesterone for a long period of time (contraceptive or hormone replacement therapy). There is normally a difference in hearing throughout life between women and men where hearing starts to decline in the thirties for men and in the fifties for women. This difference cannot entirely be explained by a difference in occupational noise exposure (39, 40). There is growing evidence for a protective effect of estrogen on hearing by BDNF,

a neuroprotective peptide, responsible for the postponed hearing decline in women (41-43). This effect might be dose- and age dependent (44-46). There are studies suggesting that menopausal women treated with Hormone Replacement Therapy (HRT) have a better hearing than the non-treated, however the results have been contradictive and some are arguing for a negative effect of the progesterone part in HRT (47, 48).

Hearing loss

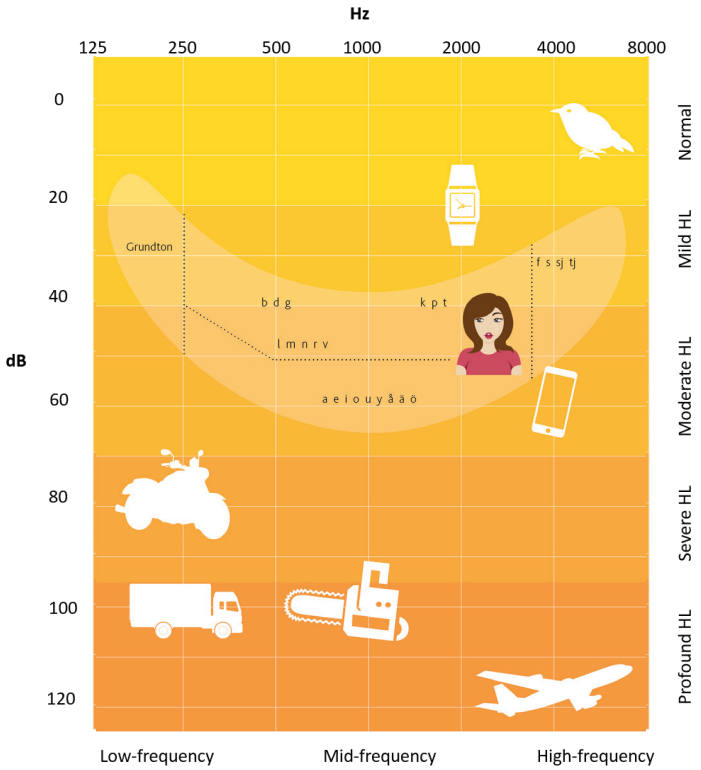

Figure 5: An audiogram showing hearing per frequency (in hertz, Hz) in decibel (dB). The grades of hearing loss are shown in different shades of yellow from normal hearing to profound hearing loss (HL). Specific environmental sounds and phonemic sounds in the Swedish language are shown in regard to the hearing level needed for detection. Published in adapted form with the kind permission of Cochlear Nordic AB.

There are three main *types* of peripheral hearing loss, conductive, sensorineural and mixed. Conductive hearing loss is due to a disturbed pathway for the sound from the ear canal and through the middle ear. Problems inter-

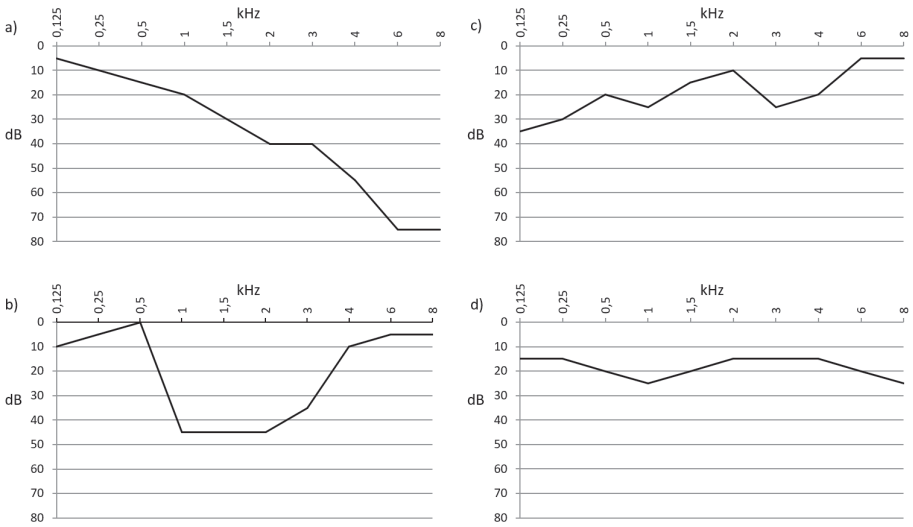

Figure 6: Types of audiometric configuration with a) high frequency sloping, b) mid-frequency u-shaped (dip), c) low-frequency rising and d) flat loss.

fering with the inner ear or nerve give rise to sensorineural hearing loss. The combination of these two is called a mixed hearing loss. As described in the preface, hearing loss can also be divided in regard to *degree* from mild to profound (figure 5) as well as by *configuration* (high-frequency sloping, mid-frequency u-shaped, low-frequency rising, flat loss) (figure 6).

Treatment for hearing loss

There are different types of treatment for hearing loss depending on the type and degree. Surgery with ossiculoplasty or middle ear implants can be efficient for mild to moderate conductive or mixed hearing loss. In other cases, a hearing aid is a better solution. For severe to profound hearing loss a cochlear implant is the treatment of choice. If the cochlea or the cochlear nerve is missing a brainstem implant is the prefered solution.

Hearing aids

The most common treatment for hearing loss today is hearing aids. It is used in mild to severe hearing loss and works as a sound amplifier, resembling the function of the middle ear. It collects the sound with an external microphone, enhances the signal and projects it into the ear canal. For this system to work, the ear must have enough hair cells remaining in the inner ear in

order to create an electric signal to the brain. When there is a lack of hair cells the possibility to hear will decrease in general, but the lack of hair cells can also affect the quality of the sound making it harder to interpret words. For people with severe to profound hearing loss a cochlear implant might be the treatment of choice.

Cochlear implant

A cochlear implant is a hearing aid that transduces sound collected by an outer microphone into electrical impulses, through the utilization of a processor. These impulses are magnetically transferred through the skin and in to the inner receiver. A thin electrode is placed in the Scala tympani in the cochlea where the neurons are stimulated directly. This permits sound to bypass non-functioning hair cells in the inner ear and send the stimuli directly to the nerve. The tonotopic function of the inner ear helps the patients to distinguish between different frequencies (figure 7). The cochlear implant was invented independently by three research groups in Australia, Austria and the USA (49). The first cochlear implant had a single channel for stimulation and only provided the possibility of hearing sounds. Today the implants have between 12-22 channels and the great majority of patients use their implants for speech perception.

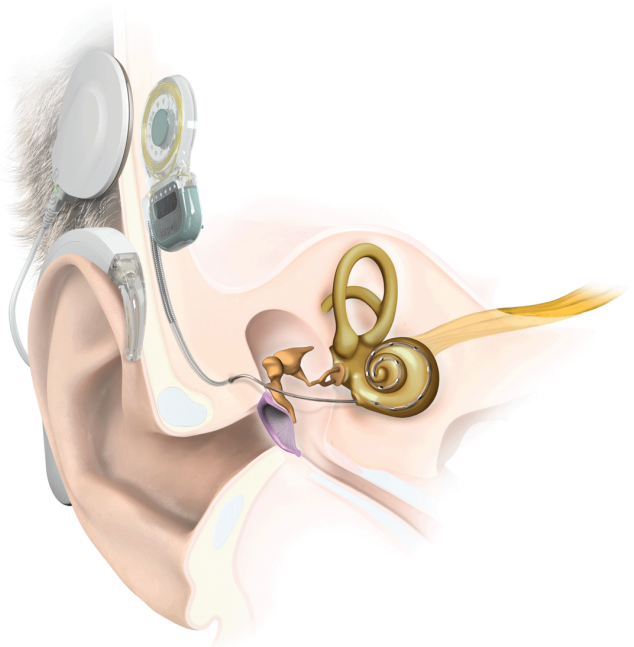

Figure 7: A cochlear implant with its outer processor transmitting the sound via the magnet through the skin to the implant on the inner side. The electrode passes via the mastoid, middle ear and through the round window in to the cochlea. Reprinted with kind permission from MedEl Nordic AB.

AIMS

The overall aim of this thesis is to enlighten the close connection between hearing and endocrinology both directly via hormonal effects on the inner ear and indirectly where endocrine syndromes have an influence on both hearing and the endocrine target organs.

Specific aims:

1. To describe the audiometric features in young women with Turner syndrome and the extent of hearing rehabilitation with hearing aids (Study I).

2. To investigate the effect of estrogen substitution, growth hormone and anabolic steroid treatment on hearing in young women with Turner syndrome. This research has been conducted using two cohorts with different treatment schemes (Study II).

3. To investigate a possible direct effect of progesterone via progesterone receptors in the inner ear (Study III).

4. To investigate the natural history of the development of hearing loss and vertigo in children and young adults with Pendred syndrome and DFNB4 and to describe the results on hearing, language and social skills development after cochlear implantation in these individuals (Study IV).

SUBJECTS AND METHODS

Subjects and design

Studies I and II

Sixty-four women with Turner syndrome between the ages of 25-38 were included from the Turner center at Karolinska University Hospital. The women were recruited from two time-frames and were born between 1973 and 1985 (n=35, mean age: 32.4 years) in the recent cohort, and from 1954 to 1967 (n=29, mean age: 32.7 years) in the historic cohort. The two cohorts were chosen to compare the effects of estrogen substitution, growth hormone, and anabolic steroid treatment before and after the introduction of a new treatment guideline 1980 (study II). In study I all subjects were pooled to describe the audiological features in this age-span of young women with Turner syndrome.

All subjects performed or had performed a pure-tone audiometry with air- and bone conduction thresholds (0.125-8 kHz and 0.5-4 kHz respectively). The type and degree of hearing loss as well as type of audiometric configuration was identified and the degree was compared to a Swedish cohort comprised of normal hearing individuals. Information about estrogen, growth hormone and anabolic steroid treatment, karyotype, otitis proneness, ear surgery, spontaneous puberty, smoking, family history of hearing loss, treatment with ototoxic medication and the use of hearing aids were collected from the patients' charts. These studies were approved by the regional ethics committee (No 2012-374-311).

Study III

In total sixteen inner ears from 3-12-month-old, male and female Sprague Dawley rats and two 60 years old postmortem donated human inner ears were used in this study. All rats were bought from the Jackson Laboratory and were housed locally in a controlled environment with food and water ad libitum. The rats were euthanized after being deeply anaesthetized with Xylazine, Ketaminol and thereafter carbon monoxide was used. The inner ears were removed and, depending on the method of investigation, decalcified, paraffin-embedded and sectioned for immunohistochemistry, dissected from surrounding bone for PCR or placed in an ice-cold solution of complete protease inhibitor for the Western blot. All animal experiments were approved by the Animal Care Committee in Stockholm, Sweden (N359/02, N370/04, N200/5 and N157/09).

The two human inner ear specimens were donated to us. Both cochleae were from 60-year old females with no previous history of hearing disease or hearing loss. The first cochlea was harvested post mortem in Austria and used for the immunohistochemistry studies. The second cochlea was harvested during skull base surgery for Schwannoma and was prepared and cryosectioned in Uppsala to be used for immunofluorescence studies. The use of the specimens was approved by local ethics committees in Austria and Uppsala (no.99398, 22/9 1999, no.C254/4, no. C45/7 2007).

Study IV

Twenty-nine individuals, aged between 1.8-25.2 years, diagnosed with LVAS and/or IP2 inner ear malformation, and implanted with unilateral or bilateral cochlear implants as children participated in the study. Five individuals had genetically diagnosed Pendred syndrome. The hearing history could be divided in three distinct subgroups: (1) severe to profound congenital deafness and early surgery (before the age of 2 years), (2) late implanted congenitally deaf children or hearing aid users with moderate to severe hearing loss, but slow progress in speech development and (3) progressive deafness with hearing aids preoperatively with a history of good hearing. They all participated in a full day of investigations including hearing tests, speech and language tests, vestibular and balance tests, and a structured medical interview. Questionnaires were distributed to parents and teachers regarding social and cognitive skills. Retrospective information regarding surgery, electrode use and adverse effects was collected from the patients' charts. MRI and/or CT scans were reviewed and systematically analyzed. This study was approved by the regional ethics committee (2014/2068-31/2).

Audiology

Pure tone audiometry and audiometric features

In order to establish the hearing level of the quietest sound detectable for an individual the normal "hearing test", pure tone audiometry, is performed in a sound proof room. Adults and older children listen to a sound in headphones and push a button when the sound is detected. For the younger children, a behavioral observation audiometry is performed when the audiologist detects a behavioral change in the child as a reaction to a given sound. Air conduction and bone conduction audiometry for the frequencies 0.125-8 kHz and 0.250-4 kHz respectively was performed in studies I, II and IV. Pure tone average (PTA4) were calculated as the mean of 0.500, 1, 2 and 4 kHz and was

used to determine the *degree of hearing loss* according to the HEAR-classification (figure 5, table 6) (50). In studies I and II, *type of hearing* was classified as: normal hearing, sensorineural-, conductive- or mixed hearing loss. Normal hearing was classified as a hearing level per frequency better than 20dB, a hearing level per frequency 20 dB or worse with a difference between bone and air conduction of less than 15 dB was classified as sensorineural hearing loss and a difference between bone and air conduction of 15 dB or more with a normal bone conduction was classified as conductive hearing loss. A mixed hearing loss had a difference of 15 dB or worse between air and bone conduction with a bone conduction worse than 20 dB. The *classification of hearing loss* was divided into five different subtypes according to the modified classification by Hederstierna et al (47). The types are high-frequency sloping, mid-frequency u-shaped, flat loss and low-frequency rising (figure 5).

	Definitions
Grade of hearing loss according to HEAR	
Normal hearing	<20 dB
Mild hearing loss	20-<40 dB
Moderate hearing loss	40-<70 dB
Severe hearing loss	70-<95 dB
Profound hearing loss	95 dB and above
Configuration	
High-frequency sloping	The average threshold at 0.5 and 1 kHz is ≥15 dB better than the average of the thresholds at 4, 6 and 8 kHz
Mid-frequency u-shaped	One or more adjacent thresholds between 0.75 and 2 kHz are ≥20 dB poorer relative to any thresholds at lower frequencies and ≥15 dB poorer relative to threshold at higher frequencies
Flat loss	Thresholds across frequencies 0.25-8 kHz do not vary more than 15 dB from each other
Low-frequency rising	The average of the thresholds at 0.25 and 0.5 is ≥15 dB poorer than the average of the thresholds at 4, 6 and 8 kHz

Table 1: Definitions of the grade of hearing loss according to HEAR (50) and the different configurations in accordance to Hederstierna et al 2007 (47).

Speech Audiometry and Adaptive HINT

Children and adults with cochlear implants usually have difficulty hearing in noisy environments (discrimination). In order to assess the level of discrimination three different methods were used in study IV: speech audiometry in quiet and noise and adaptive HINT (Hearing In Noise Test). Hearing in noise has a higher demand on the overall auditory system due to the activation of both primary auditory cortex and other auditory associated areas in the brain. Loss of function in the auditory associated areas e.g. in aging or hearing impairment will hamper the hearing in noise (51).

The participants in study IV performed speech audiometry in quiet from the age of four, where they listened to lists of twenty-five monosyllabic phonetic balanced words and were asked to repeat what they heard. The test was then performed in background noise that had a fixed signal to noise ratio of 0 dB (52). The speech and noise test was performed from the age of six. The result is presented as a percentage of correct answers. In the adaptive HINT the participants listened to lists of short phrases in which the signal to noise ratio was changed in regard to correct or false answer (53). The result is the level of background noise in dB where the individual can repeat 50% of phrases correctly resulting in a test without an upper or lower limit.

Sound-localization

Sound-localization is important for directional hearing as well as hearing in noise. Persons with hearing impairment have difficulty with both. A better result has been shown with bilateral cochlear implants when comparing with a unilateral listening situation (52). The patients' age at time of implantation and the time between implantation in the two ears also play key roles (52, 54). In this test, used in study IV, the individual were placed in front of five loudspeakers covering 180 degrees with an inter-loudspeaker spacing of 45 degrees. The patient utilized their best-hearing solution (e.g. bilateral cochlear implants; bimodal hearing aid and cochlear implant; unilateral cochlear implant) (52). A sound was presented and the individual was instructed to indicate the source of the sound. The sounds were given in a random order from the multiple loudspeakers, for ten repetitions. An error index (EI) was calculated based on the sum of all azimuth errors in the test. This resulted in a value between 0 and 1 where 0 is a perfect match and 1 corresponds to complete guessing. A result >0.540 was regarded better than chance. This test was performed from the age of four.

Laboratory tests

Sprague Dawley rats were used for all laboratory work, a model well used for mapping receptors in the inner ear.

Immunohistochemistry
This method used in study III is employed to map the existence, localization, and distribution of receptors in tissues. A standard immunohistochemistry technique (avidin-biotin-peroxidas) was mainly used for the rat cochleae and a standard immunofluorescence staining in the human specimen. These techniques utilize the antibody-antigen connection principle by using an antibody specific for the target protein in question, in this case Progesterone receptors A and B and both together (PRA, PRB, PR). In immunohistochemistry, a second antibody chemically linked to peroxidase will bind to the first and this step will make it possible to identify the protein by staining. In immunofluorescence, the second antibody is labeled by a detectable fluorescent dye.

For the immunohistochemistry, thin sections from dissected, decalcified and paraffin imbedded inner ears from twelve rats (six 3-month old females and six 12-month old males) and one human specimen were used. They were stained with the following antibodies: NCL-PGR312 for PRA, MA1-411 for PRB and MA1-410 for PR, according to the routine protocol in the laboratory (55). For negative controls the antibody was replaced by non-immune serum and tissue from rat and human uteri was used for positive and negative controls. After being incubated with the second antibody (biotinylated horse anti-mouse IgG antibody), followed by horse-radish peroxidase-avidin-biotin complex a DAB-staining for exposure times from 20-90 s was used and then counterstained with hematoxylin. The sections were inspected and photographed by using a Zeiss Axioplan microscope connected to a PixieLINK camera.

The immunofluorescence staining technique was used for enabling visualization of both the PRs and the cell nuclei in the human section. A fresh frozen, thin section was incubated with the same primary antibodies as used in the rat sections, but as second antibody Alexa 488 (for green fluorescence) and DAPI (for blue nuclei fluorescence) were used. Human uteri were used as positive and negative controls. The staining was visualized in a Zeiss LSM510 confocal microscope with appropriate filters and excitation wave-lengths.

PCR, Polymerase chain reaction

This method was used in study III in the attempt to amplify PRB mRNA from the inner ear. The DNA is denaturized by a brief heating and then cooled to allow primers to attach to its target sequence after which a DNA polymerase will make a new copy of the mRNA with the use of nucleotides in the surrounding liquid. This sequence will be repeated several times to be able to multiply even small quantities of mRNA exponentially into measurable amounts. The end-product is then controlled by using an agarose gel electrophoresis, size-separating the RNA product and the gel is stained to identify the result.

Four cochleae from two 3-month old female rats were micro-dissected from the surrounding bone and isolated using RNeasy® mini kit. After preparation, PCR was performed in a thermal cycler (2720 Thermal cycler) and the PCR products were stained with GelRed and analyzed on a 2% agarose gel.

Western blot

This is a third method used in study III in the attempt to identify PRA and PRB proteins in the inner ear. By lysing the cells, the proteins are extracted and size-separated by gel electrophoresis. With the knowledge of the protein-size (120 kD for PRB and 80 kD for PRA) the progesterone receptors can be identified after antibody binding.

Two one year old rats, in total four cochleae, one from each sex were used. The frozen tissue was homogenized in a MagnaLyser separately for the male and the female cochleae and the cytosolic fraction was separated. A nuclear extraction buffer produced the nuclear fraction after centrifugation. In order to ensure the protein-content a BSA-protein assay kit was used showing 20 µg protein in the cytosolic fraction and 10 µg in the nuclear. The proteins were then separated in a NuPAGE 4-10 % Bis-Tris gel and transferred onto a polyvinylidene difluoride membrane to enable interaction with the antibody. Transfer efficacy was controlled by a MemoCode reversible Protein stain kit. To reduce non-specific binding the membrane was placed in a blocker medium (Odyssey blocking buffer) before incubated with PR antibody (MA1-410). The second antibody (IRDye800- and IRDye680 conjugated secondary antibodies) was added before band detection was performed using direct infrared fluorescence detection. A rat uterus and GAPDH´40 (an added protein with the size of 40 kD) served as control.

Speech and language, cognitive and social skills

All tests regarding speech and language and cognitive skills in the participants were performed by an experienced Speech and Language therapist in regard to their age. Social and pragmatic skills, as well as the participants' mental health, were measured by questionnaires completed by the parents of children under the age of 18. The test-battery aimed to recognize any specific difficulties in this cohort with cochlear implants due to Pendred syndrome or DNFB4.

Speech and language tests

Several tests are regularly used to determine the speech and language level and progress for every cochlear implanted child at the Cochlear implant unit. This is performed to ensure the improvement and quick detection of a need for increased support. Speech and language tests are divided in receptive (passive) and expressive (active) knowledge and a deficit in one area or the other indicates different causes. A third part is the pragmatic skills test which determines the ability to adapt language usage to a social context. All tests have their specific age-frames which are the reason the participants were tested with different numbers of age appropriate tests.

The Peabody Picture Vocabulary test IV (PPVT IV), a picture identifying test, was used from the age of two to examine receptive vocabulary and the results were compared to American norms (56). *The Boston Naming Test* (BNT) used from the age of four is a picture naming test, designed to measure expressive vocabulary. The Boston Naming Test resulted in a raw score which was translated into a stanine compared with Swedish normative data (57). For the level of expressive grammar, the in-house scale (*Syntax*) was used with the scores 1-8 where 1 was equal to "no use of voice with intent" and 8 been equal to "typical or correct expressive grammar and sentence level" (58). The *Speech and Intelligibility Rating scale 2* (SIR2) evaluates the level of understandable speech (59). Finally, the parents answered the *Children's Communication Checklist 2* (CCC-2), a questionnaire regarding pragmatic skills in children four to fourteen years of age (60). The results were compared with English norms. A *Level of spoken language development* was determined, based on the results of all tests and test-scores within a year from age-matched normal hearing individuals were rated as age-equivalent.

Cognitive tests
Knowledge of the cognitive capabilities of a child is useful in learning situations in order to adapt the level of difficulty and in choosing exercises. Here they are used as screening tools for cognitive deficits. Two different assessments were used.

Raven colored matrices is a test for non-verbal cognitive ability for children four to twelve years of age. The score is transferred to percentiles and compared to English norms (61). A parent and teacher questionnaire, *Behavior Rating Inventory for Executive Functions* (BRIEF), was used for evaluating the executive functions from the age of two (62). Test results were compared with American norms.

Social skills
Social skills, mental health and speech and language development are closely interconnected. There is a risk, especially in children with hearing impairment, for speech and language delays which could affect social interaction. In order to screen the participants for social skills and mental health issues, the Strength and Difficulties Questionnaire was answered by parents and teachers (63). It contains 25 items grouped in five subscales: emotional symptoms, conduct problems, hyperactivity-inattention, peer problems and prosocial behavior. The score is validated in Swedish as well as for people with hearing impairment (64, 65).

Surgery

The 29 patients with LVAS and/or IP2 malformation had had a normal cochlear implant procedure at the Karolinska University Hospital after preoperative investigations regarding speech and language, hearing and vestibular function, medical assessment, electrocardiography (ECG) and imaging (MRI and/or CT scan). The surgery was performed during general anesthesia by a limited mastoidectomy and a posterior tympanotomy to reach the round window while preserving the facial nerve and corda tympany (the taste nerve). The electrode was then introduced into the Scala tympani via the round window, a cochleostomy or, in some cases, an extended round window procedure. As an extra precaution, nerve monitoring of the facial nerve is compulsory during surgery. All patients were given one dose antibiotics and cortisone in accordance to their weight during surgery. The patients stayed overnight postoperatively and had a plain x-ray for control of electrode placement the following day before leaving the hospital.

Radiology

All available MRI and CT scans were reviewed by an experienced radiologist and an ENT surgeon. The x-rays were reexamined for modiolar dysplasia, apex dysplasia, asymmetric relation between Scala vestibuli and tympani (Scalar asymmetry), vestibular aqueduct enlargement (>1.5 mm), semicircular canal anomalies and symmetrical findings between inner ears (figure 4).

Statistics

Non-parametric tests were used in studies I, II and IV due to the small number of participants and non-normal distribution of variables. Chi^2 tests were applied to determine a significant difference between two groups regarding a dichotomous outcome. For calculations where there were less than five individuals in one or more of the possible turn-outs, the Fischers exact test was used. The Mann-Whitney U-test compares ordinal data from two independent groups. If more than two groups were compared Kruskal Wallis test was applied. Binary multiple regression was used in study II for evaluation of treatment effect of estrogen substitution, anabolic steroid and growth hormone treatment on hearing adjusted for karyotype, spontaneous puberty, and otitis proneness. Significance was regarded as $p<0.05$ with *<0.05, **<0.01 and ***<0.001. All analyses were performed using SPSS version 22.

RESULTS

Studies I and II

Seventy-seven women with Turner syndrome were identified and 64 (83 %) were finally included, 35 out of 45 women in the recent group and 29 out of 32 in the historic group. Fifty-three percent had karyotype 45,X, 14 % 46,X,i(Xq) and 33 % a mosaics karyotype. The percentages in-between these groups differed in the recent and historic cohort but when comparing 45,X and 46,X,i(Xq) versus mosaics there was no significant difference between cohorts (p=0.796). Collected medical data are presented in table 1.

	45,X (%)	46,X,i(Xq) (%)	Mosaic (%)	Recent (%)	Historic (%)	All (%)	Missing (%)
n	34 (53)	9 (14)	21 (33)	35 (55)	29 (45)	64	
Mean age	32.3	32.8	32.8	32.4	32.8	32.6	
Mean age at detection	8.4	12.0	16.6	7.9	15.1	11.2	
Genotyp							
- 45,X				21 (60)	13 (45)	34 (53)	0
- mosaic				11 (31)	10 (34)	21 (33)	0
- 46,Xi(Xq)				3 (9)	6 (21)	9 (14)	0
Treatment							
- None	0 (0)	0 (0)	3 (14)	1 (3)	2 (7)	3 (5)	0
- Estrogen	34 (100)	9 (100)	18 (87)	34 (97)	27 (93)	61 (95)	0
- Growth hormone	21 (62)	3 (33)	10 (48)	33 (94)	1 (3)	34 (53)	0
- Anabolic steroids	22 (65)	7 (78)	9 (43)	23 (66)	15 (52)	38 (59)	0
Mean treatment length (year)	15.8	15.4	10.7	16.1	12.4	14.1	2 (3)
Mean estrogen score	21.9	22.2	15.2	20.1	19.4	19.8	2 (3)
Spontanous puberty	4 (12)	3 (33)	13 (62)	9 (26)	11 (38)	20 (31)	0
Otitis prone as a child	18 (53)	4 (44)	8 (38)	18 (51)	12 (41)	30 /47)	2 (3)
Otosurgery	4 (12)	2 (22	3 (14)	3 (9)	2 (7)	5 (8)	1 (2)
Hearing aid	6 (18)	1 (11)	0 (0)	3 (20)	5 (17)	8 (11)	2 (3)
Ototoxic substances	0 (0)	1 (11)	0 (0)	0 (0)	1 (3)	1 (2)	30 (47)
Family history of hearing loss	3 (9)	2 (22)	3 (14)	2 (6)	6 (21)	8 (13)	23 (36)
Smoking	3 (9)	0 (0)	3 (14)	0 (0)	6 (21)	6 (9)	8 (13)

Table 2: Collected medical data for all subjects divided in karyotype and cohort respectively.

Audiologic configuration

In general, women with karyotype 45,X and 46,X,i(Xq) suffered from poorer hearing thresholds than the group with mosaic karyotype (p=0.034). There was no significant difference between the results for 45,X and 46,X,i(Xq) regardless of type, configuration or grade. Sensorineural hearing loss was the type of hearing loss most frequently seen in the participants (45 %) and with a predominance in karyotype 45,X and 46,X,i(Xq). They also showed a higher percentage of high-frequency sloping and mid-frequency u-shaped configurations. In Figure 8, the mean hearing level divided by degree of hearing loss according to HEAR in paper I is presented. The characteristic progress in hearing loss from normal hearing with a slight mid- and high-frequency dip transforms into a more pronounced mid-frequency dip and mild hearing loss. In the group with moderate hearing loss the progress is worse in the high frequencies, resulting in a classical sloping configuration, hiding the mid-frequency dip.

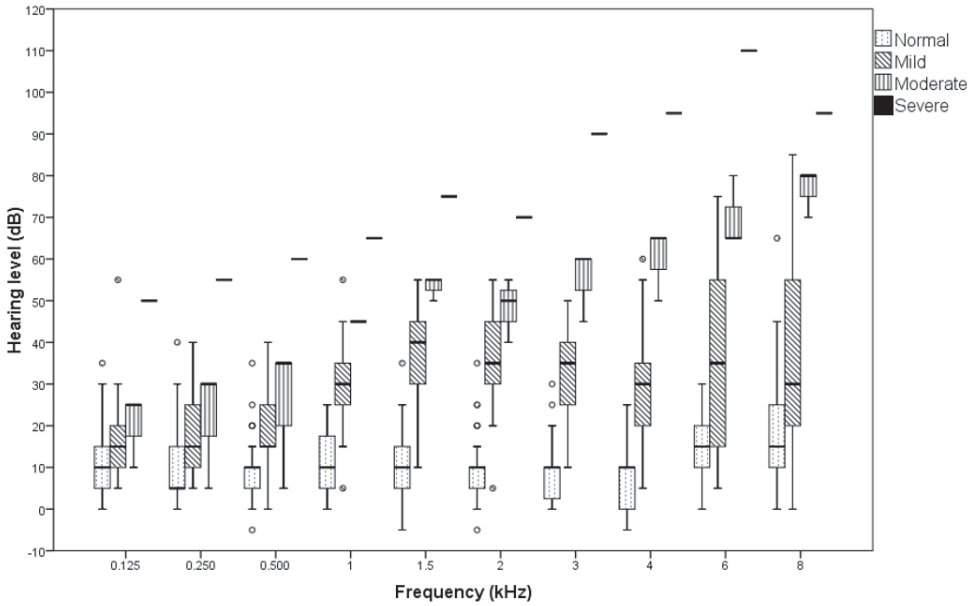

Figure 8: Median hearing thresholds per frequency and divided by grade of hearing loss according to HEAR (50).

Compared with an otologically unscreened, non-occupationally noise-exposed Swedish population of 337 women between 18-80 years of age, the women with Turner syndrome (aged 25-38) had a median hearing level, in the low and high frequencies, slightly worse than 50-59 year old women in the normal population. In the mid-frequencies (b), they resembled 60-69 year old women. Divided in grade of hearing loss according to HEAR, the normal hearing women with Turner syndrome had a hearing in level comparable to 50-59 year old women with normal hearing (a).

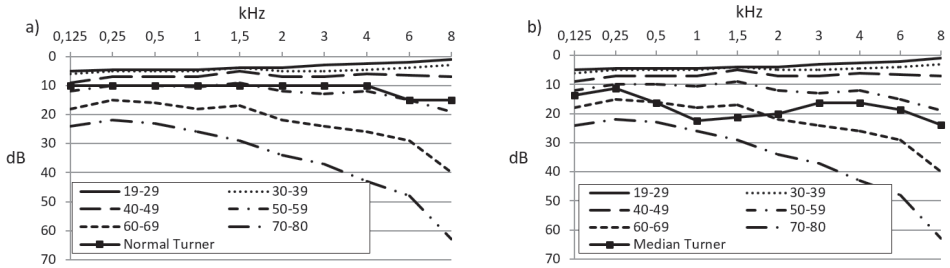

Figure 9: Median hearing in normal hearing women with Tuner syndrome (a) and the median hearing level all women with Turner syndrome (b) (all aged 25-38) compared with a Swedish cohort of non-occupationally noise-exposed women aged 18-80.

Hearing and Endocrine Treatment

There were no correlations of hearing loss per frequency, type of hearing or configuration with estrogen treatment regardless of time or dosage. Estrogen scores show no correlation, except for a negative association between estrogen score and mid-frequency u-shape configuration. Growth hormone treatment had a significant negative association with sloping configuration and hearing loss in the high frequencies independently of karyotype, otitis media proneness and spontaneous puberty (figure 10). Anabolic steroid treatment had a significant positive association with sloping and a positive correlation with otitis proneness.

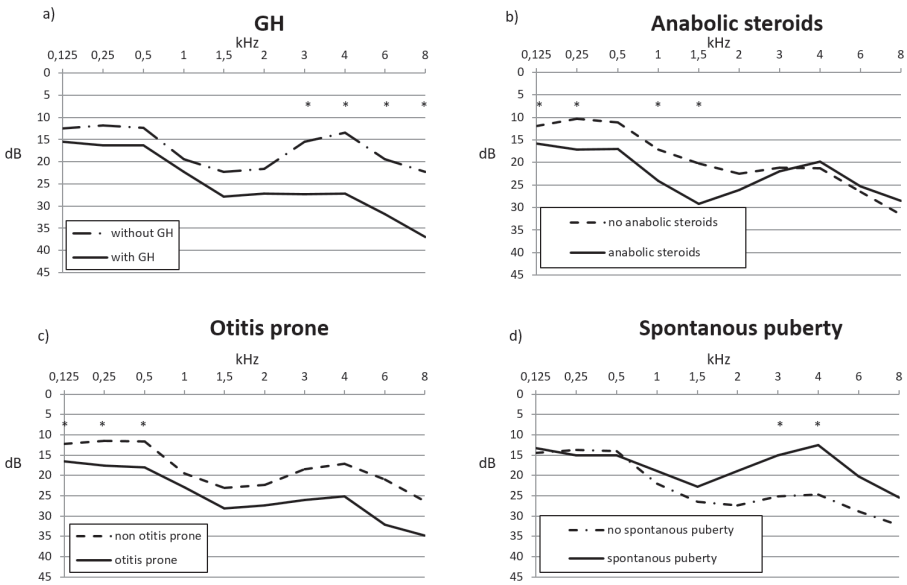

Figure 10: Mean hearing thresholds in women with Turner syndrome with and without GH treatment a), anabolic steroid treatment b), otitis proneness c) and spontaneous puberty d). A statistically significant difference (*) was found in univariate tests (Mann-Whitney U-test) regarding GH treatment at 3-8 kHz, anabolic treatment at 0.125-0.25 and 1-1.5 kHz, otitis proneness at 0.125-0.5 kHz as well as spontaneous puberty at 3-4 kHz.

Cohort and Hearing

A significant difference was identified in the prevalence of normal hearing (p=0.036) and risk for sloping configuration (p=0.017) between the cohorts with less normal hearing and more sloping configuration in the recent cohort. Regarding back-ground factors there were significantly more women with growth hormone treatment (p=0.000), estrogen treatment for a longer period (p=0.001), but with lower total estrogen score (p=0.024), and fewer number of smokers (p=0.007) in the recent cohort.

Hearing aids

Eight women out of 64 (12.5 %) were already rehabilitated with hearing aids at the time of inclusion in the study and with a hearing ranging from mild to severe according to HEAR (50). Two women had at least one ear with a severe hearing loss and were both fitted with hearing aids, however, only about one third of women with moderate hearing loss were fitted. All of them had sloping or mid-frequency u-shaped configuration.

Study III

Immunohistochemistry/Immunoflourescence

There was no visible staining of PRA, PRB or PR in the organ of Corti, stria vascularis or spiral ganglion in rats or humans after repeated staining. In the surrounding cochlear bone, a nuclear staining of both PRB and PR could be identified. A weak cytoplasmic staining of PRB in large ganglion cells was seen in both paraffin embedded and fresh-frozen human samples but not in

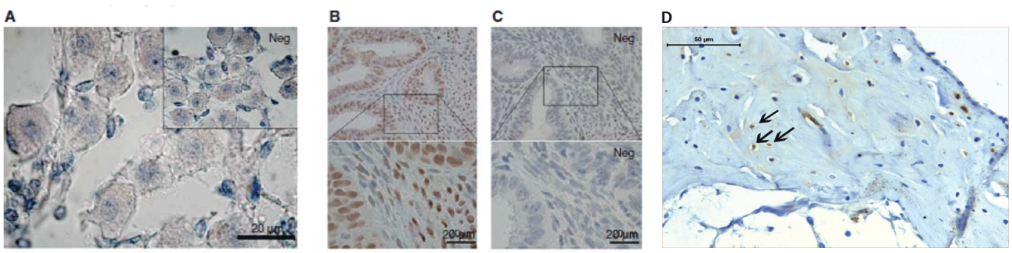

Figure 11: Immunohistochemistry for progesterone receptor A (PRA) showing no nuclear staining in spiral ganglion in human inner ear (A) and a positive nuclear staining in endometrial controls (B) but none in negative controls (C). A positive nuclear staining for progesterone receptor B (PRB) in surrounding bone (black arrows) in rat inner ear is shown in figure D).

the rat inner ear. A shade of this was noted in human specimens using the PR antibody. The positive and negative controls showed a correct nuclear staining pattern in regard to their treatment except for PRA in rats were the antibody did not show any staining neither in the inner ear nor the endometrial controls, e.g. the PRA antibody was non-functioning. Therefore, we used a PR antibody staining both PRA and PRB at the same time, in order to investigate if PRA could be present in the inner ear. The results were negative, showing no nuclear staining in the inner ear.

PCR

The PCR was only performed in rat due to a lack of human specimen. There was a negative result on the PCR for PRB in the rat organ of Corti, stria vascularis and spiral ganglion confirming the results of the immunohistochemistry.

Western blot

For minimizing protein loss, the whole rat cochlea, as well as bone, was included in the Western blot. PRB could be detected in both nuclear and cytoplasmic fractions in male and female rats, but there was no detection of PRA. This concludes the findings of PRB in the surrounding cochlear bone and a lack of PRA in the inner ear.

Study IV

Twenty-nine patients, all of whom underwent cochlear implant surgery as children between 1991 and 2016, were included in this study and divided in three groups in regard to their hearing history. This was done in an attempt to sort out some of the problems due to a significant difference in demographic data regarding age, age at implantation, history of hearing performance, hearing aid use and schooling. This is in part due to historical differences in postoperative rehabilitation. One child with a neuropsychiatric disorder was excluded from statistical calculations. The definition of groups and demographics regarding number, sex, age and hearing history are presented in table 3. Eighty-six percent used spoken language as their primary language and 45 % were fluent in sign language probably reflecting the different historic conditions for children with severe to profound hearing loss in Sweden, where sign language was the main language for deaf children for decades. Fifty-two percent had bilateral cochlear implants, 7 % had unilateral with one deaf ear and 41 % had bimodal solutions (a hearing aid in one ear and a cochlear implant in the other). Forty-one percent attended mainstream school and as many as 23 % were or had been attending deaf,

signing schools. In the structured medical interview, there were two unexpected findings regarding general behavior. Fifty-nine percent of the participants had or had had sleeping disturbances such as difficulty falling asleep or repeatedly waking up at night. Sleeping disturbances were not correlated to particularly poor results in speech and language tests. Difficulty concentrating was reported by 52 % and this group had significantly lower score on the expressive vocabulary tests.

	Total	Group 1	Group 2	Group 3
Nb	29	8	12	9
Sex (F/M)	13/16	3/5	6/6	4/5
Median age	8.7 (1.8-25.2)	3.7 (1.8-12.1)	9.4 (3.9-25-2)	11.9 (6.9-15.3)
Median age at implant				
- first	3.1 (0.4-12.6)	0.9 (0.4-1.9)	3.1 (2.0-6.0)	5.1 (1.8-12.6)
- second	2.5 (0.7-13.3)	1.5 (0.7-2.3)	4.1 (2.5-11.0)	12.0 (10.7-13.3)
Congenital deafness	10 (34)	7 (88)	3 (25)	0 (0)
Early progress (<2 years)	8 (28)	1 (12)	6 (50)	1 (11)
Slow progress (>2 years)	11 (38)	0 (0)	3 (25)	8 (89)
Hearing mode				
CI + CI	15 (52)	7 (88)	6 (50)	2 (22)
CI + HA	12 (41)	1 (12)	4 (33)	7 (78)
CI + deaf	2 (7)	0 (0)	2 (17)	0 (0)
School	22	3	10	9
- mainstream	9 (41)	2 (67)	2 (20)	5 (56)
- special unit	8 (36)	0 (0)	4 (40)	4 (44)
- deaf school	5 (23)	1 (33)	4 (40)	0 (0)
Language				
- spoken language as primary	25 (86)	7 (88)	10 (83)	8 (89)
- fluent in sign language	13 (45)	3 (38)	6 (50)	3 (33)

Table 3: Background information regarding hearing history, schooling and language in the 3 groups and together. Group 1 = congenital or early progressive hearing loss with early implantation (<2 years of age). Group 2 = congenital deafness with late implantation (>2 years of age) and progressive hearing loss with preoperatively unsatisfying hearing aid use. Group 3 = children with progressive hearing loss and preoperatively good speech and language development. One participant in group 1 had a neurodevelopmental disorder and was not included in the results of the speech and language tests. The range is added between brackets for age and percentage between brackets for hearing history schooling and language. F = Female, M = Male.

Surgery and adverse events

In total 46 surgeries were performed including two reoperations, one due to tip fold-over of the Nucleus CI532 electrode and one due to a failing implant. Due to repeated occasions with tip fold-over or near events also outside the study, CI 532 is judged not to be appropriate for implantation in patients with LVAS and IP2. Twenty individuals used implants from MedEl and nine from Cochlear. A round window insertion was performed in 34 cases without any complications regarding insertion. An oozing (mild CSF-leakage from the inner ear) was reported in 21 of 30 (70 %) surgeries but the status was unknown in 16 cases. Ten cases had nausea (22 %) and vertigo was seen in five patients (11%) after surgery. In all but two cases the postoperative nausea or vertigo did not prevent discharge from the ward the day after surgery. No antiemetic drugs other than cortisone are given routinely during surgery. One patient had to stay another night due to a suspicion of CSF-leakage which was treated conservatively with bedrest. No CSF leakage could be confirmed.

Radiology

	Nb	%	Missing	Group 1 (missing)	Group 2 (missing)	Group 3 (missing)
Aqueduct enlargement	26/26	100	1	7/7 (1)	10/10	9/9
Vestibular enlargement	25/27	93	0	8/8	9/10	8/9
Symmetric malformation	23/26	88	1	7/8	9/10	7/8 (1)
Modiolar dysplasia	22/27	81	0	7/8	8/10	7/9
Apex dysplasia	9/13	69	14	4/5 (3)	3/5 (5)	2/3 (6)
Scalar asymmetry	8/26	31	1	4/8	4/10	0/8 (1)
Semicircular canal anomaly	7/27	26	0	5/8	2/10	0/9

Table 4: The number and percentage of inner ear malformation and the rate of inner ear malformation in each group. Group 1 represents congenital or early progressive hearing loss with early implantation (<2 years of age). Group 2: congenital deafness with late implantation (>2 years of age) and progressive hearing loss with preoperatively unsatisfying hearing aid use. Group 3 includes children with progressive hearing loss and preoperatively good speech and language development.

X-rays from 27 participants were possible to retrieve while three had a CT performed only, seven had a MRI only and seventeen had both a CT and an MRI. Eighty-nine percent (n=24) of the participants presented with LVAS bilaterally, 7 % (n=2) unilaterally and one was indecisive. IP2 malformation was present in 81 % (n=22), all bilaterally. Further radiological details are presented in table 4. There were no associations between the radiological findings and speech and language outcomes. Scalar asymmetry and semi-

circular canal anomaly were associated with a negative effect on speech recognition in quiet independent of age and were significantly more common in group 1 than group 3 (p=0.035, p=0.011).

Hearing

The mean speech recognition scores were 74 % in quiet and 34 % in noise with a significantly better result in group 3 (late implanted with history of good hearing) than in group 2 (late implanted with insufficient hearing for speech and language development) in quiet (p=0.004). There are no significant differences between groups regarding speech recognition in noise, adaptive HINT or sound localization. Participants with a history of fluctuation in hearing had a significantly higher score on speech recognition in both quiet and noise (p=0.003, p=0.046), adaptive HINT (p=0.028), bimodal hearing (0.037) and a higher incidence of sleep disturbances (p=0.005). Only one participant out of 13 had a negative speech-to-noise ratio on the adaptive HINT test compared with 70 % of normal hearing children aged 6-11 years.

Speech and language

Sixty-one percent had speech and language abilities estimated as age-equivalent or better including all in group 3 but less than half in group 2. The group presented weak results regarding expressive vocabulary but normal results on receptive vocabulary. All results in spoken language abilities were significantly correlated with speech recognition in quiet but not in noise (p=0.000-0.006). There was a generally low total score in the 19 participants who concluded the pragmatic skills questionnaire (CCC-2) with a particularly low score on the questions regarding “speech”. Participants attending a special unit or deaf school had significantly lower scores on the questions regarding “semantics” (p=0.030) as well as on expressive vocabulary (p=0.043). Participants with reported motor problems during childhood had a significantly negative effect on the receptive vocabulary test (PPVT) (p=0.009) and the expressive vocabulary test (BNT) (p=0.020) when corrected for age.

Cognitive function and social skills

The overall results for executive function, non-cognitive skills and social skills were normal. Participants with fluctuation in hearing and hereditary predisposition for dyslexia had significantly higher scores on emotional problems graded by their parents (p=0.012, p=0.007). Participants with hereditary predisposition for dyslexia also had significantly more problems on the parental total score and hyperactivity-inattention behavior (p=0.010, p=0.025) but this was not supported by the teacher scores which indicated problems at home but not in school.

DISCUSSION

Hearing in two endocrine syndromes

Endocrine systems have a strong interaction with hearing and the development of the inner ear. In Turner syndrome, as in Pendred syndrome and DFNB4 there is a vulnerability of the inner ear closely connected with the syndrome. Young adults with Turner syndrome already have a hearing situation on par with women at least twenty year older, in a Swedish cohort, and the need for hearing rehabilitation is high from an early age. The estrogen substitution does not seem to improve hearing although the combination with progesterone might interfere with the results. The pathway for progesterone interference on hearing is still unclear since we could not demonstrate a presence of nuclear progesterone receptors in the inner ear of rat and human. Significantly worse hearing in the high frequencies was seen in growth hormone treated women but other environmental factors cannot be excluded. In Pendred syndrome and DFNB4, a severe to profound hearing loss is seen. This hearing loss often has an early onset leading to a need for hearing rehabilitation with a cochlear implant. The speech and language results show low results in expressive vocabulary and pragmatic skills and vertigo, fluctuating hearing, motor problems and sleeping disturbances were common.

Hearing in Turner syndrome, Pendred syndrome and DFNB4

In Turner syndrome, the hearing problems are divided in three stages with childhood otitis-dependent conductive hearing loss, mid- and/or high-frequency sensorineural hearing loss starting as a mild type in youth and progressing to mild-severe with age. In study I the rates of conductive (1.6 %) and mixed (6.3 %) hearing loss were in the lower range compared to other studies in the same age-span where the rate was 3- 8 % for conductive and 4-24 % for mixed hearing loss (23, 66). This could be due to a lower percentage of otitis proneness (47 %) in the current study. Even though conductive and mixed hearing loss was sparse, 52 % of the women had hearing loss in at least one ear. The only other study published, by Verver et al, concentrating on women in a similar age-span showed a hearing loss in 66 % but that cohort had a higher incidence of otitis media and middle ear surgery that might explain the difference (66). The most common configurations were high-frequency hearing loss and mid-frequency dip both most frequent among women with karyotype 45,X and 46,X,i,(Xq). This is in coherence with the results from King et al and Beckman et al (23, 67).

Compared to the mild to moderate, progressive hearing loss in Turner syndrome, hearing in Pendred syndrome is regarded as mainly profound, bilateral, and congenital (31, 38, 68, 69). After the introduction of genetic testing, the phenotype in Pendred syndrome has started to be questioned due to different penetration within families regarding severity of hearing loss, thyroid enlargement, and the results on the perchlorate discharge test (a test of the organification of iodine in the thyroid decreased in Pendred syndrome) (68). Regarding this, the distinction between cohorts presented in the literature with Pendred syndrome, IP2 malformation, Mondini malformation, DFNB4 and Large Vestibular Aqueduct syndrome is somewhat blurred, obstructing the possibility to compare the results. In the cohort of children in study IV, 10 had a congenital deafness and 19 had progressive hearing loss with 11 children presenting at least one normal ear in early childhood screening showing that a majority of hearing loss is not present at birth. Of the five patients with genetically confirmed Pendred syndrome only one had congenital deafness which is a result well representative of the diversity of the subject population. A fluctuation in hearing is often a precursor to a progressive hearing loss common in patients with DFNB4. The risk of postponed diagnosis of a severe hearing loss due to uncertainty regarding the hearing results in children with LVAS and IP2 malformation is an important clinical knowledge.

Anatomical influences on hearing

In Turner syndrome, the lack of the growth-promoting SHOX-gene on the X-chromosome responsible for the short stature, also affects the development of the skull base (15, 17, 25, 70-72). This influences the placement and development of the Eustachian tube that is suggested to be the reason for the increased risk of otitis media and negative middle ear pressure. The incidence of otitis media is between 61-82 % and conductive hearing loss is common during childhood and adolescence (16, 17, 73). No immunological deficits have been determined to be responsible for the increased rate of middle ear infection (74). The frequency of tympanic membrane retraction and cholesteatoma forming is elevated in women with Turner syndrome resulting in the need for surgical intervention and a risk for an iatrogenic negative effect on hearing (19).

The hearing loss in Pendred and DFNB4 is related to an inner ear malformation in contrast to Turner syndrome where the inner ear is usually normally configurated (15, 70). Even though a cochlear malformation, in addition to LVAS, gives the impression of a more severe malformation Ahadizadeh et al could not find any statistically significant difference in hearing between the two groups (69). In study IV a relationship between scalar asymmetry and

semicircular canal anomaly and speech recognition in quiet was found as well as a higher incidence of these malformations in the group with early implantation. This might reflect a relationship between the severity of the malformation, occurrence of congenital deafness and hearing outcome which has never before been presented. The numbers are small in the study, and further work is needed to show if this is a preoperative, predictive factor regarding hearing outcome.

Genetics

Both Turner syndrome and Pendred syndrome/DFNB4 are accompanied by a wide spectrum of phenotypes. The degrees of hearing loss, vertigo, tinnitus, fluctuating hearing and goiter (Pendred syndrome/DFNB4) differ as do the associated characteristics of short stature and autoimmune disorders in Turner syndrome. In Turner syndrome the degree of the loss of the X-chromosome is more or less in parallel with the severity of symptoms with 45,X having considerably more comorbidities than mosaics as a group (12). On an individual level however, the phenotype penetration can vary which is why medical advice based on karyotype has to be carefully planned.

In Pendred syndrome and DFNB4 the association with mutations in the SLC26A4-gene is strong but differs throughout the world with a higher incidence in Asian than in Caucasian populations (75, 76). The anatomical range from an isolated Large Vestibular Aqueduct to an IP2-malformation with or without an enlarged thyroid gland necessary for the criteria of Pendred syndrome seems not closely associated with karyotype even though homozygote mutations in the SLC26A4-gene have a higher prevalence for Pendred syndrome in some studies (75-77). It is suggested that Pendred syndrome should be identified as a subtype of a SLC26A4-based syndrome with a specific pattern of penetrance instead of an isolated syndrome (78). However, in both Turner and Pendred syndromes the karyotype alone is not enough to explain the phenotype in each individual which is the reason why other factors, genetic and environmental, must be considered.

Mouse models

To further study the impact of the genetic characteristics and the genesis of the symptoms mouse models of both Turner syndrome and Pendred syndrome have been developed. The Turner mouse, 39,X is a strain with growth retardation, otitis proneness and early hearing loss in the high frequencies. It shows a loss of outer hair cells, most pronounced in the basal turn needed for high frequency hearing, and a reduced number of nerve endings connecting to the inner hair cells starting at the age of one year (79). The elec-

tron microscopic imaging also showed lateral wall disintegration as well as a swelling in the vascular stria, signs that increase with age. The decrease in hearing thresholds in the higher frequencies on ABR followed the visual findings. The reason for these findings is yet unknown.

The Pendred knock-out mouse (SLC26A4 null mouse) is profoundly deaf, shows a vestibular dysfunction and has an enlargement of the endolymphatic sac and duct as well as a cochlear hydrops (high fluid pressure) (32). The mouse model has helped in establishing the function and necessity of the Pendrin protein responsible for maintaining the acid-base balance of the endolymph. This balance is essential for creating the polarization in the hair cells necessary for the neural activity and signaling. A lack of Pendrin creates an acidification of the endolymph due to a reduced amount of bicarbonate secretion normally used to buffer the proton excess. There is an increased oxidative stress in the organ of Corti impairing the expression of KCNJ10 in the stria vascularis. The KCNJ10-gene is responsible for a potassium-regulating channel necessary for the endocochlear potential. An impaired expression of the channel explains the inability of the hair cells to produce a nerve signal in response to audiological stimuli (8). Pendrin is located both in the cochlea and the vestibular organ in the ear, which could explain the effect on both hearing and balance. It is also found in the thyroid and kidneys.

Hearing rehabilitation

With a hearing loss in the high frequencies as in Turner syndrome, most of the fricative consonants (s, tj, f, k) in the Swedish language are lost leading to a difficult listening situation. This in combination with a mid-frequency dip affecting many of the vowels increases the difficulty identifying words (Figure 6). Despite this, only eight women out of 64 in study I used a hearing aid while two out of three with moderate hearing loss did not (80). Girls and women with Turner syndrome use hearing aids to a higher extent than the rest of the population (13, 17, 73), but regarding the results in the study I the process of hearing rehabilitation could be more active in order to prevent social isolation, difficulties at work and increased disability due to hearing (81).

In Pendred syndrome and DFNB4 there is a wide range of hearing loss from one-sided moderate to bilaterally profound. This range demands different rehabilitation solutions, from hearing aids in mild to moderate hearing loss to cochlear implants in bilaterally severe to profound hearing loss. The cohort presented in study IV is concentrated on children with cochlear implants, a subgroup of this diagnosis.

Cochlear implants in LVAS and IP2-malformation

Cochlear implantation in LVAS and IP2-malformation has been described as a safe procedure despite the risk for oozing of cerebrospinal fluid upon opening the cochlea (82). In order to prevent continuous leakage and meningitis, a tight soft tissue seal should be used around the inserted electrode regardless of a round window insertion or a cochleostomy. In study IV three children had to stay another night in the hospital due to a suspicion of cerebrospinal fluid leakage in one case and severe vertigo in two cases. In contrast with the results from Mey et al the presence of oozing/gusher was common in study IV but did not seem to have a relationship with post-operative vertigo. The presentation of the postsurgical vertigo rate is sparse in the literature. In the study from Mey et al a postoperative rate of vertigo of 22 % was presented, i.e. higher than the 11 % in study IV (83, 84). On the other hand no rate of nausea was described in their study, whereas in our material some young children with vertigo might have been classified as suffering from nausea. In two studies of 300 and 475 non-malformed pediatric cochlear implant patients respectively, only two children were reported to have had postoperative vertigo showing that this complication in children without malformation is unusual (85, 86). The risk of vertigo after surgery is increased thus the patient and parents should be informed of the risk. Further examination of the results in search for possible prognostic preoperative factors will be performed.

Speech and language results

The general conclusion in study IV showing normal results on the receptive vocabulary test, but surprisingly low results on the expressive vocabulary and pragmatic skills tests that is not previously reported. In the literature regarding children with LVAS with or without IP2-malformation and cochlear implantation, there are few reported results on speech and language tests and the diversity of tests used are extensive, preventing a comparison between studies. Pritchett et al compared isolated LVAS with children with LVAS and IP2-malformation showing a non-significant advantage for the isolated LVAS group (87). Regarding hearing tests, Ahadizadeh et al have shown the same difference with worse, but not statistically significant results for the LVAS and IP2-malformation group, although the groups were small in both studies (69). The lack of a control-group in our study prevents comparison with other cochlear implanted children at our clinic. However ongoing studies will improve this matter in the near future.

Speech and language acquisition depends on several factors where hearing, attention and concentration, social skills, and motor performance all are im-

portant parts. The cohort in study IV presented a lower median score on speech recognition in quiet compared with other studies regarding children with the same malformation (69, 88, 89). This might mirror the high grade of attendance to deaf, signing schools and special units where sign language is being used to varying degrees. Only 49 % were integrated in mainstream schools and 50 % were fluent sign language users, thus bilingual. Multilingual, normal hearing children show the same pattern with lower results on expressive vocabulary, but normal on receptive vocabulary, a fact possibly explained by the reduced exposure to each language with less repetition (90). Neither sleeping disturbances nor fluctuating hearing had a negative effect on vocabulary or pragmatic skills in our material.

A heredity predisposition for dyslexia and parent-reported difficulties with concentration were associated with a sub pathologic score on hyperactivity-inattention. Parent-reported difficulty to concentrate was also associated with lower scores on the expressive vocabulary and pragmatic skills tests. No child was diagnosed with dyslexia or hyperactivity disease, e.g. ADHD, to our knowledge. Hearing impairment might lead to difficulties with concentration and hyperactivity, but the high frequencies of these problems in this study are intriguing and needs further investigation. Meanwhile, establishing a personalized speech, and language learning environment is crucial.

Motor performance is an important factor in early communication development. The ability to correspond to facial expressions, as well as interact with others, is dependent on mobility and the possibility to focus on interaction instead of movement (91). A possible competition between the focus on motor activity and hearing is also possible. In participants reporting childhood motor problems an association with decreased results in speech and language outcome was seen and is to our knowledge not reported elsewhere. This indicates a necessity to screen for motor problems and assure appropriate intervention, when needed, in order to improve the possibilities for the best hearing outcome. Further analysis regarding vestibular function and motor skills are currently ongoing in an attempt to verify these findings.

Hormonal treatment and hearing

Most articles in the literature show an overview of several periods in the lives of women with Turner syndrome. The present studies (studies I and II) concentrate on hearing in early adulthood focusing on hearing during that period of life and the evaluation of long term follow up after medical treat-

ment. There is a significant difference in hearing between the recent and historic cohorts showing a decrease in hearing thresholds over time with a reduction in the number of normal hearing individuals as well as a higher risk for sloping configuration. This can be due to an inclusion bias caused by a higher rate of normal hearing women in the historic cohort while the normal hearing individuals in the recent cohort chose not to participate in the study. Normal hearing individuals are less likely to have been tested regardless of timeframe why this should reduce the risk for bias. There is a overrepresentation of women with karyotype 45,X in the recent cohort compared with the historic cohort, however the relationship between karyotype 45,X and 46,X,i(Xq) versus mosaic karyotype is comparable in the two cohorts. In the literature, a general decrease in hearing among the younger population in Sweden has been found, especially in women, and the reason for this is not known (1). There can therefore be a general cause for worse hearing in the recent cohort but changes in treatment, cohort composition or combinations of these are other options.

Estrogen

The reason for hearing loss in Turner syndrome is yet unknown but one of the theories is based on the lack of estrogen. Estrogen receptors have been mapped in the inner ear (92, 93) and in the central auditory pathways (46) and mice lacking the estrogen receptor beta become deaf within a year of age (42). Women have quite stable hearing up until the age of menopause in comparison with men whose hearing starts to decline already around the age of 30 (39, 40). This is thought to be due to a protective effect of estrogen. In women with Turner syndrome, with an insufficient endogenous production of estrogen, the hearing decline starts early.

The cohorts in study II show differences in hormonal treatment with a shorter period of estrogen treatment, but at higher doses in the historic cohort compared to the recent cohort, and growth hormone treatment almost exclusively in the recent cohort. The difference in estrogen treatment did not show any significant association with hearing in multiple regression analysis except for a negative association between estrogen score and the presence of a mid-frequency dip. This result was regarded as non-specific due to the small cohort size since neither the length nor amount of estrogen treatment showed equal results and the result of the multiple regression model was not significant. The progesterone portion of the treatment could have an interfering impact on the hearing result and will be discussed further on.

Estrogen substitution does not start until the time for induction of puberty around the age of 12 and it is arguable that this is too late for hearing preservation. In a recently published study by Ros et al hearing in three cohorts, women with Turner syndrome, women with congenital hypogonadism due to other reasons and women with normal estrogen production, was compared (94). All groups were treated with combined hormonal replacement therapy. The women with congenital hypogonadism due to a cause other than Turner syndrome had hearing in level with women with normal estrogen levels (20 % vs 27 %) while the women with Turner syndrome had a higher percentage of individuals with hearing loss (87 %) indicating a minor role for estrogen as a cause of hearing loss. There was however a 60 % rate of spontaneous puberty in the cohort with congenital hypogonadism compared to 20 % in the Turner cohort thus the groups are not comparable in regard to estrogen deficiency.

Another theory put forward is the Cell cycle delay hypothesis based on a deficiency in the SHOX-gene situated on the short arm of the X-chromosome. This gene is necessary for the normal development of the otic capsule in a dose-dependent manner (25). This together with a generally longer cell cycle for abnormal cell lines, as 45,X, could lead to a lower density of sensory hair cells in the inner ear causing a higher sensitivity for hair cell loss that could explain the findings in the inner ear of the Turner mouse(25, 79).

Growth hormone

There was a significant negative association between growth hormone treatment and sloping configuration (high-frequency hearing loss) in study II. A worse PTA4 in a growth hormone treated group of women with Turner syndrome compared with a non-treated group has been published in the literature (23), but the reason for non-treatment was not accounted for why. This could be dependent on karyotype or other factors known to interfere with hearing. In the present study, all women were treated with growth hormone regardless of karyotype after the introduction of the new guidelines, except two individuals who were identified too late for treatment. The relationship between the two karyotypes with the highest frequency of hearing loss (45,X and 46,X,i(Xq)) and the mosaic karyotypes were equal in the recent and historic groups reducing the risk for interference on hearing due to karyotype.

Growth hormone and its metabolite IGF-1 are necessary for cell proliferation, cochlear ganglion maturation and regulation of cochlear development (95). In syndromes associated with IGF-1 deficiency hearing loss is a common but not mandatory. Symptoms range from normal hearing to profound

hearing loss (96). There is a correlation between a low concentration of IGF-1 in blood and a higher risk for hearing loss as well as otitis proneness in women with Turner syndrome (25). There is also a negative association between intrauterine growth retardation and a short final statue and sensorineural hearing loss in men (97). All these findings show the importance of growth hormone and IGF-1 in hearing and cochlear development. The relation between excessive growth hormone and hearing is less well described. Individuals with acromegaly (98) and McCune-Albright syndrome (99) have an increased risk for hearing loss that can be mixed, sensorineural or conductive. The genesis of this can be due to the excess of growth hormone but other contributing factors cannot be excluded. In conclusion, there is sparse support in the literature regarding a possible negative effect of growth hormone treatment on hearing loss. Other factors may affect these findings and further research is needed.

Progesterone

It is arguable that study II did not exclusively investigate the effect of estrogen on hearing in Turner women given that the therapy most often used is a combination of estrogen and progesterone. There are studies showing a negative effect of progesterone on hearing as part of Hormone Replacement Therapy in menopausal women where both pure-tone testing, DPOAE (Distorsion Product OtoAucoustic Emissions) and hearing in noise tests were worse in combined treatment groups as compared to estrogen treatment alone (48, 100). Although there are indications of an effect of progesterone on the inner ear the pathway is yet unknown.

Experimental studies

Immunohistochemistry is a non-quantitative measure and problems can be encountered at various levels. The inner ear is a challenging organ due to its composition of delicate soft tissue enclosed by the densest bone in the body. The decalcification procedure, the antigen-retrieval and the specificity and sensitivity of the antibodies can all interfere with the results. Insufficient blockage of non-specific proteins can cause background staining and an obscuring of the results. The antibodies used in study III had all been successfully used before in different tissues. Regardless, the PRA antibody did not show any staining neither in rats nor in humans on both the test and control samples. The reason for this is unknown and adjustments in the test-procedure with additional washing and adjusted denaturation processes did not change the outcome. In order to ensure coverage of the PRA-receptor with the immunohistochemistry method the PR antibody with an affinity to both receptor A and receptor B was used as well.

PCR and Western blot are quite robust methods, but they are sensitive to impurities in the sample and it is important to have a good amount of the protein to measure. In PCR, the primer needs to be specific enough to bind only to the DNA selected for amplification but short enough to be efficient. If the primer reacts with other parts of the DNA the output will be non-specific and if it is too long the number of amplifications will be reduced and the result might be falsely negative. For Western blot as for Immunohistochemistry the specificity and sensitivity of the antibodies are important as are the characteristics of the gel. In a gel with a bigger pore size the proteins will travel faster and vice versa which necessitates that it is chosen in regard to the protein of interest.

In study III, no nuclear receptors in the organ of Corti, stria vascularis or spiral ganglion were found either by immunohistochemistry or PCR. In Western blot PRB receptors were found and the immunohistochemistry confirmed the presence of nuclear PRB in the surrounding bone. The lack of PRA in the Western blot confirmed the lack of PR staining in the rat inner ear's immunohistochemistry. All the results were coherent for male and female rats as well as humans including the different ages in rat.

Other possible pathways

Regarding the negative effect of progestin treatment on hearing, the impact is probably mediated via other pathways. Progesterone has both nuclear receptors, transmembrane receptors as well as membrane-associated receptors, the last two acting via signaling cascades at the cell membrane level (101). No mapping of the latter two has been made in the inner ear thus their existence is unknown. Progesterone is also a known antagonist to the mineralocorticoid and corticoid receptors preventing their activities. These receptors are known to be present in the inner ear, both in stria vascularis, where they are responsible for the ion hemostasis, and in the hair cells (102-104). Progesterone has an especially high affinity to the mineralocorticoid receptor; a receptor used by corticosteroids and, above all, the mineralocorticoid aldosterone. Aldosterone regulates the potassium balance in the body and interacts with the ion homeostasis in the inner ear necessary for depolarization of the hair cells. In the aging human, the concentration of aldosterone normally decreases and a correlation between low serum aldosterone levels and a higher incidence of high frequency hearing loss has been observed. Aldosterone substitution in a mouse model showed a protective effect on hearing due to a blocking effect of apoptotic pathways improving the cell survival in the spiral ganglion cells, particularly in the basal turn of the cochlea (105). Treatment with progesterone containing medication could

inhibit the effect of aldosterone thus increasing the risk for high-frequency hearing loss.

Thyroid hormones

Thyroid hormones are crucial for the early neuromotor development as well as for the development of the inner ear (106). In congenital hypothyroidism, early treatment with thyroid hormone substitution will reduce the risk for severe hearing impairment as well as the negative effect on mental, motor, language and cognitive development (107). There are three known thyroid receptors, one thyroid receptor alpha and two thyroid receptor beta (1 and 2). Mouse models deficient in the different thyroid receptor beta receptors show different responses. In thyroid receptor beta 1 deficient mice a profound hearing loss is developed whereas the hearing seems to stay intact in thyroid receptor beta 2 deficient mice (108, 109). In Pendred syndrome the thyroid hormone levels are within normal limits in at least 50 % of the individuals, but some develop a hypothyroidism in need for substitution (31, 38). The Pendrin protein is involved in the organification of iodine in the thyroid gland, however it is not the sole anion exchanger responsible for this process (34). The reduced expression of Pendrin in SLC26A4 mutations can lead to a deficit, or subclinical deficit, in thyroid hormone levels that might interfere with the neuromotor development, possibly contributing to the associated symptoms regarding behavior, motor development and language acquisition seen in study IV. Further research regarding the effect of a subclinical thyroid hormone deficiency on neuromotor development is needed.

Limitations

The retrospective character of the studies on women with Turner syndrome always has its limitations regarding the possibility to retrieve information from the charts and uncertainty regarding accuracy in treatment-time, coverage of participants and medical issues. The possible negative interference of the progesterone compound in the estrogen treatment might affect the results regarding hearing. Further measures to calculate the progesterone portion could possibly have clarified the hormonal effect on hearing. However, the effects of progesterone on hearing, as well as the pathway, are not clear; therefore the possibility to estimate the effect of different compounds would be difficult.

In the aim to cover both sexes and a wide age-range, male and female rats (3, 6 and 12 months of age) were used although with different methods

leaving a possibility of a changing receptor activity in different sexes and ages. Additional Western blot tests with a dissected cochlea and at different ages would have ensured the lack of progesterone receptors in the inner ear with higher accuracy. Nevertheless, the consistency of the experiments, throughout both sexes and ages, strengthens the findings.

In the study of cochlear implants in children with LVAS and/or IP2 malformation one important weakness is the lack of genetic confirmation of Pendred syndrome and DFNB4. This leaves a possibility of including participants with other syndromes where these malformations are present. All participants except one have ongoing genetic testing, thus future work on this cohort will include these results.

The decision to include all patients with LVAS and/or IP2 malformation having received a cochlear implant as children leads to a diverse group regarding age at time for testing as well as their representation of different rehabilitation schemes over the years. The children operated in the 90-ties and early 2000 had sign language education to a higher extent than children operated today. A between subject difference also exists in regard to regional traditions in postoperative rehabilitation. The addition of a control group would also have strengthened the study.

FURTHER PERSPECTIVES

- The effect on hearing by the progesterone portion in combined contraceptives needs to be investigated to exclude an additional reason for the worse hearing in the recent cohort
- To understand the negative effect of progesterone on hearing, mapping of the progesterone receptors in the central auditory pathway as well as membrane receptors and indirect pathways are essential.
- Further insight in vestibular and balance function in children with LVAS and IP2 and its relationship to hearing and speech as well as language outcome would be an important contribution for the improvement of the rehabilitation in these children.
- Sleep disturbances are common in children with LVAS and IP2 and significantly impact family life. Further investigation of sleep-pattern, presence of obstructive sleep apnea and the impact on family life would improve the possibilities for preventive programs to assist in the improvement of sleep patterns.
- Further investigation of the presence and frequency of radiological findings, particularly scalar asymmetry and semicircular canal anomaly, in LVAS with IP2 malformation and its relationship to the outcome and genetics.
- The rate of and predictive factors for adverse events after cochlear implantation in patients with Pendred and DFNB4 need to be further evaluated.
- The relationship between the significantly poor results in speech and language development in children with parent-reported concentration difficulties needs further investigation in order to enhance the support in language acquisition. A possible link to brain development in Pendred syndrome has to be further investigated.

CONCLUSIONS

1. Study I: Sensorineural hearing loss is common in young adults with Turner syndrome and the two most frequent types of hearing loss are mid-frequency u-shaped and high frequency hearing loss. Hearing in young women with Turner syndrome deteriorates early and is devastating for the listening situation. Young women with Turner syndrome would benefit from a more active hearing rehabilitation regimen. It is important with an implementation of a continuous control of the hearing as well as early contact with counselling. Rehabilitation, when needed, should begin as early as possible in order to ensure the possibility for social well-being and active participation in the working environment.

2. Study II: Estrogen treatment does not seem to have any positive effect on hearing, but the progesterone content might interfere with the results. An association with high frequency hearing loss in growth-hormone treated women has been detected and may reflect a negative effect on hearing by growth hormone treatment.

3. Study III: There is no direct effect of progesterone via nuclear progesterone receptors in the organ of Corti, stria vascularis or spiral ganglion but Progesterone receptor B is present in the surrounding cochlear bone. The progesterone effect is probably mediated through other pathways, the transmembrane-bound, membrane associated progesterone receptors, mineralocorticoid- or glucocorticoid receptors.

4. Study IV: Children with Pendred syndrome and DFNB4 deafness with LVAS and IP2 malformation are a complex group with weak results on expressive vocabulary and pragmatic skills tests. A history of affected motor skills and parent-reported concentration difficulties seems to be a risk factor which explains why it is important to screen for these difficulties. Semicircular canal anomalies and Scalar asymmetry might be a preoperative predictor for a worse outcome.

POPULÄRVETENSKAPLIG SVENSK SAMMANFATTNING

Omkring 5 % av världens befolkning är drabbad av hörselnedsättning som påverkar deras vardag enligt WHO. Vid många endokrina syndrom finns även en påverkan på hörsel och vid både Turner syndrom och Pendred syndrom är hörselrehabiliterande åtgärder med hörapparat respektive cochleaimplantat vanligt förekommande. Man har även sett effekter av hormonell behandling på hörseln där progesteroninnehållande hormonbehandling i menopaus har associerats med sämre hörsel.

Målet med denna avhandling är att belysa förhållandet mellan hörsel och endokrinologin både genom studier av endokrina syndrom samt genom att undersöka vägar för direkt hormonell påverkan på innerörat.

I studie I och II har hörseln hos unga kvinnor med Turner syndrom studerats samt östrogen-, tillväxthormon- och anabola steroid-behandlingens eventuella effekt på hörseln studerats. Sensorineural hörselnedsättning, framförallt i form av försämrad hörsel i diskanten och i mellanregistret, är de vanligast förekommande hörselkonfigurationerna. Grad av hörselnedsättning är oftast mild till måttlig och leder till behov av hörselrehabilitering med hörapparat i större utsträckning än för normalbefolkningen. Ingen effekt kunde ses på hörseln av östrogenbehandling men en försämrad diskanthörsel sågs i gruppen behandlad med tillväxthormon.

I studie III kartlades förekomsten av progesteron receptor A och B i innerörat på råtta och människa i avsikt att identifiera en möjlig väg för direkt påverkan av progesteron på innerörat och hörseln. Inga progesteron receptorer kunde identifieras i hörselbildande strukturer i innerörat vid immunohistokemi, PCR eller Western blot. Dock påträffades Progesteron receptor B i snäckans omgivande ben.

I studie IV kartlades den preoperativa hörselutvecklingen hos barn och unga med cochleaimplantat och Pendreds syndrom eller DFNB4 med inneröremissbildning i form av LVAS och/eller IP2. Även det hörselmässiga och språkliga resultatet utvärderades. En stor bredd i den preoperativa hörselutvecklingen sågs från kongenital dövhet till en långsamt progressiv hörselnedsättning, ofta med fluktuerande inslag. Språkmässigt hade deltagarna normalt passivt ordförråd men nedsatt aktivt ordförråd samt pragmatisk förmåga. En förhöjd andel av deltagarna hade även yrsel, fluktuerande hörsel, påverkad motorisk utveckling, nedsatt koncentrationsförmåga samt sömnstörningar.

Sammanfattningsvis så är hörselnedsättning ett vanligt problem i samhället och är inte sällan kopplat till endokrina syndrom, t.ex. Turner syndrom och Pendred syndrom. Båda dessa syndrom kan leda till grav hörselnedsättning även om kongenital dövhet endast är förknippat med Pendred syndrom. Tidiga rehabiliterande insatser med hörapparat eller cochleaimplantat beroende på hörselnedsättning är nödvändig för att motverka social isolering samt gagna språkinlärning och taluppfattning. Hormonell behandling kan ha en påverkan på hörseln men effekten av progesteron sker troligen via andra vägar än direkt via nukleära receptorer i innerörat.

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