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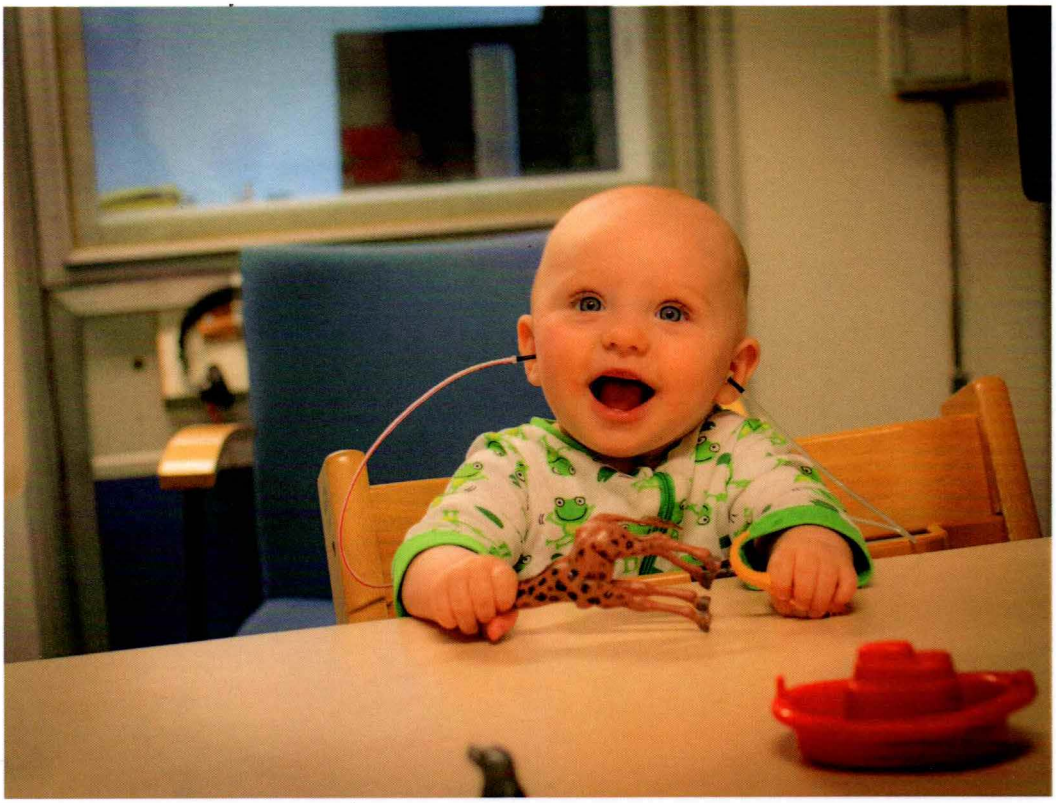
Children with Congenital Unilateral Sensorineural Hearing Loss-Etiology, Newborn Diagnostics, and Hearing Aid Amplification



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CHILDREN WITH CONGENITAL UNILATERAL SENSORINEURAL HEARING LOSS— ETIOLOGY, NEWBORN DIAGNOSTICS, AND HEARING AID AMPLIFICATION

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By

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To my beloved Erik, Vilhelmina and Assar

"All we have to decide is what to do with the time that is given us."

J.R.R. Tolkien, The Fellowship of the Ring

"If I had more time, I would have written shorter."

Variants attributed to Blaise Pascal, Marcus Tullius Cicero & Mark Twain

Popular science summary of the thesis

Why are children born with hearing loss in one ear? And how do we best help children with lifelong hearing loss in one ear? I started the PhD project with the purpose of answering these two questions.

It has been known since the 1980s that children with hearing loss in one ear, unilateral hearing loss (uHL), struggle in school. Several studies found that children with uHL often had to repeat school years. In one study 35% of children with uHL had repeated a school year, compared to 3.5% of their peers (Bess & Tharpe, 1986). The struggle in school is probably connected to various problems children with uHL may experience. It is often hard for them to hear where sounds are coming from. It is typically also hard for them to follow conversations when it is noisy, especially when several people are speaking at the same time. If you recall school, a lot of situations are noisy! However, the problems vary greatly between different children with uHL and research is incomplete to predict who will struggle and who will not.

We designed our first study to investigate if hearing aids (HAs) help school-aged children born with lifelong uHL. We studied the most common lifelong type of uHL affecting the inner ear and/or the hearing nerve. It is known as unilateral sensorineural hearing loss (uSNHL) and is the most difficult uHL to help children with, as it cannot be treated, or improved with surgery.

In the first study we evaluated the HAs children with uSNHL used in their everyday lives. We found that the HAs helped in some test environments, but not in others. The children expressed that the HAs helped them follow conversations with few people. But when people were talking in the background, the HAs neither helped nor hindered them from following conversations. This, according both to the children and the results of our tests.

When using their HAs, the children generally had a harder time localizing where sound was coming from, compared to without their HAs. We then tried to understand why the HAs did not help them hear where the sound was coming from. We found a relationship between the poor results with the HAs and the brain's response to sound ("the ear's EEG"). The longer it took for sound to travel from the inner ear to the brainstem, the poorer the result. Abnormal brain waves along the hearing nerve means less neural activity. And too little neural activity is often improved with hearing technology that improves the acoustic signal, like a HA or implant. But the hearing technology needs to be introduced early in development, as the nerve with its synapses needs constant input to function well. All children in the study started using HAs after the age of 5, which may be too late in development. We considered the large differences in results between the children and returned to the first question in the PhD project: why a hearing loss exists in the first place.

Can the hearing loss cause be connected to the large differences in HA benefit and the problems experienced?

We started exploring the reason for hearing loss by studying the test used to identify uSNHL. The transient-evoked otoacoustic emission (TEOAE) is used as a first step to identify hearing loss in all newborns in Sweden, and most newborns in Europe. The TEOAE shows similarities to "an echo" that is recorded in the ear canal in response to sound if the inner ear function is normal. With a fundamental research design, we studied the TEOAE responses of twins to find out how much the TEOAE response is explained by genes. If the response is not entirely inherited, it may be influenced by other body functions, like hormone exposure. The newborn TEOAE results were almost entirely explained by genes. For newborns who were not related, there were large differences in their TEOAE responses. Thus, our study supported the metaphor that TEOAEs can be likened to fingerprints, as they vary greatly between individuals and are largely inherited.

In the final two studies we explored why hearing loss exists in one ear for 20 infants born with uSNHL and described the hearing profiles. We invited all infants born with uSNHL (i.e., congenital uSNHL) in Region Stockholm to the study from 2019-2020.

In contrast to infants with hearing loss in both ears, most of the infants with uHL had abnormal structures in the hearing system seen with imaging. We could not see a hearing nerve on the impaired side in 50% of the infants, and 29% had abnormal or missing inner ears.

Surprisingly, none of the infants had a positive newborn cytomegalovirus (CMV) infection test. CMV infection is common and can cause hearing loss if the mother is affected for the first time during pregnancy. Previous studies have shown that CMV is common in cases of uSNHL, but not for congenital uSNHL specifically. Thus, congenital CMV infection may be an uncommon cause for congenital uSNHL. However, CMV spread varies over time and the Covid-19 pandemic may have contributed to less infection exposure during the study years.

Genetic tests have previously been rather unsuccessful in diagnosing uSNHL. To address this issue, we used more extensive genetic test panels that screen for a larger number of genes and restricted our group to children with congenital uSNHL. Five out of 18 infants (28%) received a genetic diagnosis. All three children with co-morbidities observed at birth, that is, additional malformations (of the heart, brain, hands and/or anus), received a genetic diagnosis.

Lastly, the hearing tests that target neural activity (the auditory brainstem responses and the acoustic reflex thresholds) did not show signs of what we refer to as loudness recruitment. Loudness recruitment is typically reported by adults with SNHL. Therefore, it is unusual that we did not see this neural pattern for children with congenital uSNHL. A

person with loudness recruitment hears soft sounds poorly, but when sounds get stronger the person perceives them as loud as a person with normal hearing. The neural test of loudness recruitment has been linked to adults with loudness recruitment (as measured by asking how they perceive sound). It is impossible to ask neonates and small children about how loud they perceive sounds, so neurological tests are better suited.

In summary, we recommend imaging of the auditory system for infants born with uSNHL and genetic testing for suspected non-syndromic uSNHL, as the cause for uSNHL is then often found.

Children with uSNHL can get both benefit and dis-benefit from HAs introduced late in development. Our results together with previous studies of animal models indicate that HAs may be more efficient if fitted earlier in development. However, research of actual early HA outcomes in children with uSNHL is needed to help in clinical decision making.

Loudness recruitment is an important factor to consider when fitting HAs. Loudness recruitment needs further study in older research participants with congenital uSNHL enabling the use of different hearing tests, to confirm whether most children with uSNHL have an absence of loudness recruitment.

How to best help children with uSNHL is a broad area of study. Children with uSNHL show large differences in etiology, that presumably have large effects on hearing device outcomes. Thus, our results indicate that an understanding of the connection between etiology and hearing device outcomes would help clinical decision making.

Abstract

Congenital unilateral sensorineural hearing loss (uSNHL) comprises about 25% of the sensorineural hearing losses (SNHLs) found through newborn hearing screening (NHS) programs. Even if children with congenital uSNHL struggle in school and everyday listening situations, studies on etiology, hearing aid (HA) outcomes and intervention are few, so it is still unknown when and how intervention is optimally provided.

The overall aim of the PhD project was to study the causes and mechanisms underlying congenital uSNHL and the effects of intervention. The four studies describe effects of HA amplification on pediatric congenital uSNHL (**Study I**), a basic research study of the transient-evoked otoacoustic emission (TEOAE, **Study II**), and causes for congenital uSNHL and affected auditory mechanisms (**Studies III and IV**).

In **Study I** six school-aged children with congenital uNSHL were studied. They all had HA experience and were fitted with HAs late in development. Outcomes showed both HA benefit and dis-benefit. HA benefit was found in one-to-one communication, whereas dis-benefit was found for sound localization accuracy. A close relationship was found between aided sound localization and neural maturation. In **Study II** neonatal TEOAE heredity was studied in 454 twins, showing that TEOAE levels are largely inherited. Neonatal female twins with male co-twins did not show masculinized (i.e., reduced) TEOAE levels, contrary to the twin testosterone transfer hypothesis proposed previously based on young adult twin's OAEs. **Studies III and IV** investigated etiology in 20 infants with congenital uSNHL, consecutively recruited from the newborn hearing screening (NHS) program in Region Stockholm. Malformations were found in 64% of the 14 infants tested with imaging, 50% showed no cochlear nerve on the impaired side, and 29% showed inner ear malformations. All 20 infants tested negative for congenital cytomegalovirus (cCMV) infection. The interaural acoustic reflex threshold and auditory brainstem response (ABR) results indicated a lack of loudness recruitment. Of the 18 infants that were genetically tested, 28% received a genetic diagnosis. All three infants with comorbidities observed at birth received a genetic diagnosis, whereas 13% (n = 2/15) of the infants without comorbidities observed at birth received a genetic diagnosis.

The overall results indicate that congenital uSNHL is different from bilateral SNHL, with many malformations, different auditory mechanisms, and a less explored genetic workup. Based on the results we recommend imaging for all congenital uSNHL, and genetic testing for alleged syndromic congenital uSNHL, due to high diagnostic yields. Late-fitted HAs can give both benefits and dis-benefits to school-aged children depending on the listening situation. Finally, HAs may be more efficient if loudness recruitment differences may be taken into account in the HA fitting to children with congenital uSNHL, and if HAs were fitted earlier in development, although this needs to be specifically evaluated in future research.

List of scientific papers

I. **Johansson, M.**, Asp, F., & Berninger, E. (2020a). Children With Congenital Unilateral Sensorineural Hearing Loss: Effects of Late Hearing Aid Amplification-A Pilot Study. Ear and Hearing, 41(1), 55-66.
II. **Johansson, M.**, Olofsson, Å., & Berninger, E. (2020b). Twin study of neonatal transient-evoked otoacoustic emissions. Hearing Research, 398, 108108.
III. **Johansson, M.**, Karltorp, E., Edholm, K., Drott, M., & Berninger, E. (2022). A Prospective Study of Etiology and Auditory Profiles in Infants with Congenital Unilateral Sensorineural Hearing Loss. Journal of Clinical Medicine, 11(14), 3966.
IV. **Johansson, M.**, Karltorp, E., Asp, F., & Berninger, E. (2023). A Prospective Study of Genetic Variants in Infants with Congenital Unilateral Sensorineural Hearing Loss. Journal of Clinical Medicine, 12(2), 495.

Publications not included in this thesis

I. Rance, G., Saunders, K., Carew, P., **Johansson, M.**, & Tan, J. (2014). The use of listening devices to ameliorate auditory deficit in children with autism. The Journal of Pediatrics, 164(2), 352-357.

Contents

List of abbreviations

aABR	Automatic auditory brainstem response
ABR	Auditory brainstem response
ABR threshold	ABRthr
APHAB	The Abbreviated Profile of Hearing Aid Benefit
ART	Acoustic stapedius reflex threshold
BN	Background noise (APHAB subscale)
BOR	Branchiootorenal (syndrome)
cCMV	Congenital cytomegalovirus
CHARGE	Coloboma, heart defect, atresia choanae, retarded growth and development, genital and ear abnormality
CHILD	Children's Home Inventory for Listening Difficulties
CI	Cochlear implant
CMV	Cytomegalovirus
CROS	Contralateral routing of the signal
CT	Computed tomography
DBS	Dried blood spot
DSL	Desired Sensation Level
DPOAE	Distortion product otoacoustic emission
DZ	Dizygotic
EC	Ease of communication (APHAB subscale)
EI	Error index
EVA	Enlarged vestibular aqueduct
HA	Hearing aid
HL	Hearing loss
IE	Impaired ear
IHC	Inner hair cell
I/O	Input/output (function)
MORL	Molecular Otolaryngology and Renal Research Laboratories

MRI	Magnetic resonance imaging
MZ	Monozygotic
NE	Normal hearing ear
NH	Normal hearing
NHS	Newborn hearing screening
OAE	Otoacoustic emission
OHC	Outer hair cell
OME	otitis media with effusion
OS	Opposite-sex
PCR	Polymerase chain reaction
PTA	Pure-tone average
PTT	Pure-tone threshold
PEACH	The parents evaluation of aural/oral performance of children
RECD	Real ear to coupler difference
RV	Reverberation (APHAB subscale)
SECDI	Swedish Early Communicative Development Inventory
SFOAE	Stimulus frequency otoacoustic emission
SII	Speech intelligibility index
SLA	Sound localization accuracy
SNHL	Sensorineural hearing loss
SNR	Signal-to-noise ratio
SOAE	Spontaneous otoacoustic emission
SRT	Speech recognition threshold
SS	Same-sex
SSD	Single-sided deafness
TEOAE	Transient-evoked otoacoustic emission
uHL	Unilateral hearing loss
uSNHL	Unilateral sensorineural hearing loss

1 Introduction

Hearing loss (HL) is found in every country and region worldwide. About 20% of the global population live with HL according to the World Health Organization (WHO, 2022). Almost all congenital HL is identified already a few days after birth through universal newborn hearing screening (NHS) programs. Congenital HL is one of the most common chronic birth anomalies, with a prevalence of approximately 1 in 500 births (Berninger & Westling, 2011; Bussé et al., 2020; Mehl & Thomson, 1998; Morton & Nance, 2006).

The PhD project focuses on the congenital unilateral sensorineural HL (uSNHL), with a prevalence of approximately 1 in 1500-2000 births (Berninger & Westling, 2011; Bussé et al., 2020; Mehl & Thomson, 1998; Morton & Nance, 2006). Children with uSNHL have unilateral HL (uHL), affecting one ear. It is also sensorineural, affecting the sensory organ (i.e., the inner ear), and/or the cochlear nerve. Finally, the focus is on congenital uSNHL, meaning that the uSNHL is present from birth.

Children with uSNHL have an increased risk of experiencing academic difficulties, psychosocial challenges, and speech-language delays, compared to children with normal hearing (NH) (Kuppler et al., 2013; Lieu, 2013; McKay et al., 2008). However, the outcomes have been mixed and based on rather small, heterogenous groups of children with uSNHL (Huttunen et al., 2019). Children with uSNHL generally have impaired speech understanding in noise and competing speech (Bess et al., 1986; Bovo et al., 1988; Johansson et al., 2020a; Ruscetta et al., 2005), and impaired sound localization accuracy (SLA) (Bess & Tharpe, 1984; Bess et al., 1986; Humes et al., 1980; Johansson et al., 2020a; Newton, 1983). These outcomes also vary between children with uSNHL.

Clinical decisions regarding diagnostic methods to find the uNSHL etiology are mostly based on retrospective studies of uSNHL, and children with congenital uSNHL diagnosed comparably late (around 4 years of age) (Masuda et al., 2013; Nakano et al., 2013; Orzan et al., 2021; van Beeck Calkoen et al., 2017). Genetic causes for uSNHL are also sparsely evaluated (Gruber et al., 2017; Paul et al., 2017; Sloan-Heggen et al., 2016; van Beeck Calkoen et al., 2019), and selective sampling is often an issue for generalizing the results. The late diagnosis ages (3-7 years of age) also make it difficult to determine if the comparably low incidence of genetic causes were due to many cases of non-congenital uSNHL, even when the apparently acquired losses were excluded. Thus, prospective studies of consecutively recruited infants with congenital uSNHL would be of value.

The outcomes of HA intervention show heterogeneous results in small groups of children with uSNHL (Benchetrit et al., 2022; Briggs et al., 2011; Johansson et al., 2020a; Johnstone et al., 2010; Rohlfs et al., 2017; Updike, 1994).

The aim of this thesis was to study and describe pediatric congenital uSNHL with focus on the causes and affected auditory mechanisms, and the effects of intervention with hearing aids (HAs).

2 Literature review

2.1 The auditory system

The first processing of sound takes place when sound, caused by vibrations in air, is picked up by the auricle (Figure 1). The vibrations are transmitted to the ear drum that, via the auditory bones, sets the fluids in the inner ear in motion through the oval window of the inner ear (e.g., Roeser et al. (2007)).

The inner ear comprises the cochlea and the vestibular system (Figure 1). The cochlea is about 3.5 cm long and is formed like a cone-shaped spiral staircase that makes ~2.75 turns (Figure 2) (Gelfand, 2009).

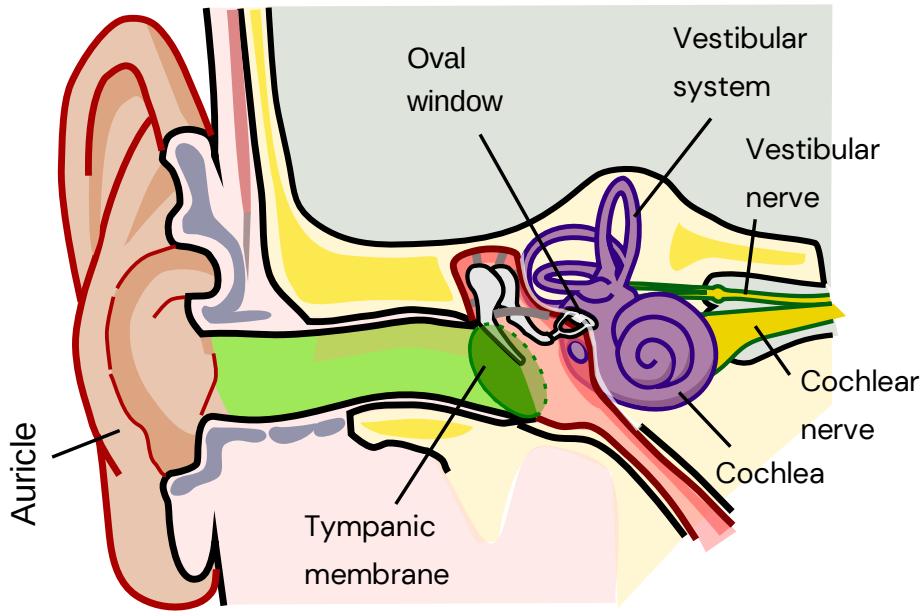

Figure 1. "Anatomy of the human ear". Illustration by Lars Chittka; Axel Brockmann, CC BY 2.5, via Wikimedia Commons, only text has been slightly modified.

The cochlea consists of three separate fluid-filled chambers: scala vestibuli, scala media and scala tympani. The organ of Corti is in ductus cochlearis, i.e., scala media (Gelfand, 2009) (Figure 2 and close-up in Figure 3).

The cochlear amplifier acts through hair cells situated on the basilar membrane in the organ of Corti. The hair cells transduce the auditory signal through hair bundle sub-micrometer deflections, where the stereocilia on top of the hair cells contain mechanoelectrical transducer channels (Fettiplace, 2017). The stereocilia are connected by tip-links that become tense in response to a calcium-driven motor that activates the mechanoelectrical transducer channels (Fettiplace, 2017). A place-frequency map exists along the basilar membrane partition, the higher the frequency the shorter the distance the travelling wave has to travel to reach its peak (von Békésy & Wever, 1960).

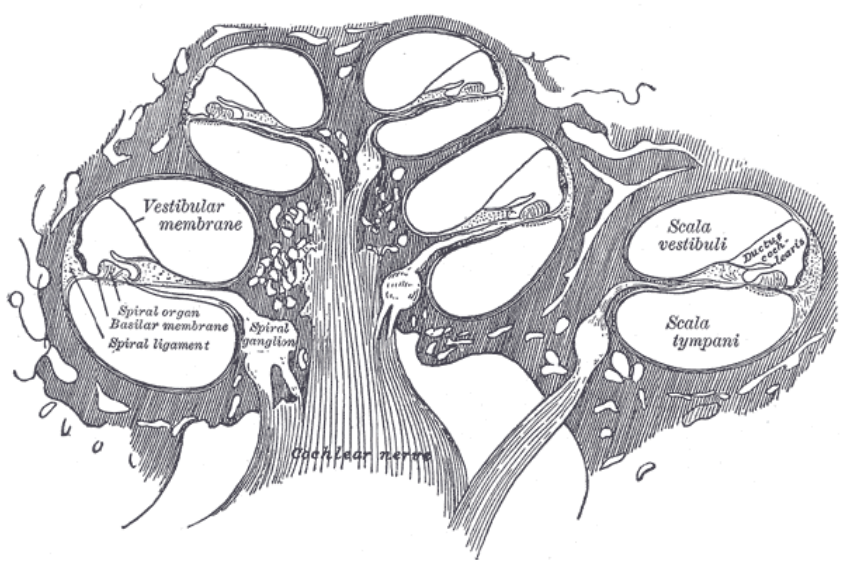

Figure 2. Cross section of the cochlea with its ~2.75 turns. The scala media is identified as ductus cochlear to the right in the figure. Illustration By Henry Vandyke Carter - Henry Gray (1918) Anatomy of the Human Body. Bartleby.com: Gray's Anatomy, Plate 928, Public Domain via Wikimedia Commons.

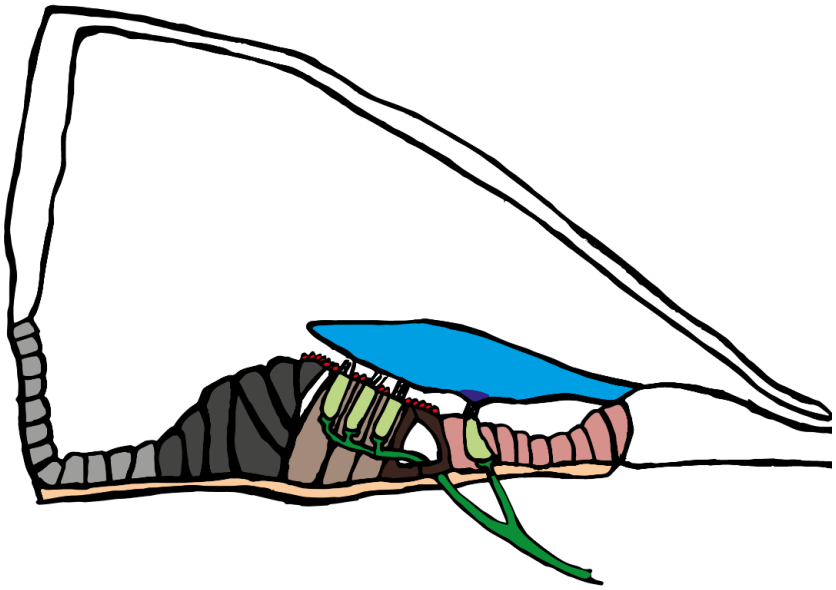

Figure 3. The organ of Corti as structured in the basal parts of the human cochlea with three rows of outer hair cells, and one row of inner hair cells (light green). The hair cells are placed on the basilar membrane (orange). Illustrated are also the tectorial membrane (blue) with Hensen's stripe (dark blue), and the beginning of the cochlear nerve (dark green). The reticular lamina is marked in red above the pillar cells (dark brown), Deiters' cells (light brown) and Hensen's cells (dark grey). Claudius' cells (light gray) and the border cells of inner sulcus (pink/light rose) are also shown. Illustration by Marlin Johansson inspired by the Figure 2 of Fettiplace (2017) and Roeser et al. (2007).

The ~12 000 outer hair cells (OHCs) are arranged in three distinct rows in the basal part turn of the cochlea (light green to the left in Figure 3), whereas incomplete four to five

rows exit in the apical parts (Bredberg, 1968; Gelfand, 2009; Wright, 1984; Wright et al., 1987). It is almost exclusively the single row of ~3 500 inner hair cells (IHCs, light green to the right in Figure 3) that are responsible for conveying the acoustic information as electrical signals to the cochlear nerve (Figure 2 and Figure 3). The OHCs add instantaneous gain to the stimulus by electromechanical motility (Brownell, 1983), known as the active process (Davis, 1983).

The first peripheral part of the auditory system, illustrated in Figure 1, is situated within the temporal bone. When electric impulses are transmitted from the cochlea to the cochlear nerve, the auditory signal exits the peripheral system and enters the central nervous system. The cochlear nerve and the vestibular nerve exit through an opening in the temporal bone known as the internal auditory canal (Gelfand, 2009). The nerves pass through the internal auditory canal together and form the eight cranial nerve, the vestibulocochlear nerve. The nerves go separate ways when the cochlear nerve enters the cochlear nuclei. The cochlear nerve then transmits the auditory information, with several cross-overs to the opposite side, via the brainstem to the auditory cortex (Gelfand, 2009).

The first part of the auditory system from the pinna to the oval window of the inner ear is known as the conductive system, as its main function is to lead (conduct) sound to the cochlea (Gelfand, 2009). A conductive HL is a HL in this part of the auditory system. A sensorineural HL (SNHL) is an impairment in the sensorineural system, i.e., the inner ear or in the neural parts of the auditory system (e.g., (Roeser et al., 2007)).

The perceptual consequences of HL are different for SNHL and conductive HL. SNHL is always associated with reduced audibility. If the inner ear is affected, the SNHL may also result in reduced dynamic range of hearing (including loudness recruitment), reduced frequency selectively, and poor temporal resolution (Dillon, 2012; Moore, 1996, 2008). The consequences lead to degraded speech understanding, especially in noisy listening environments (Leek & Summers, 1996; Moore, 2008). Conductive HL is associated with only reduced audibility, which typically makes intervention with HAs more successful, as amplification can restore audibility if enough sound is conducted to the inner ear (Dillon, 2012).

2.2 Diagnosing congenital uSNHL

A uHL can be due to impairment in various parts of the auditory system. This includes the outer ear (e.g., atresia), the middle ear (e.g., otitis media with effusion), or the inner ear (e.g., enlarged vestibular aqueduct, EVA, or cochlear aplasia) (Johansson et al., 2022; Roeser et al., 2007). It can also be due to impaired synapses between the IHCs and the subsequent cochlear nerve (e.g., auditory neuropathy), along the cochlear nerve (e.g., cochlear nerve hypoplasia or vestibular schwannomas) or in the cortical parts of the brain (e.g., brain injury) (Johansson et al., 2022; Rance & Starr, 2015; Roeser et al., 2007).

A uHL can be in the conductive part of the auditory system (conductive uHL) or in the sensorineural part of the auditory system (uSNHL). This thesis concentrates on uSNHL. Children can be born with congenital uSNHL, or acquire uSNHL throughout development (e.g., through meningitis) (Muzzi et al., 2019). Some children with congenital syndromes (e.g., due to cytalomegavirus, CMV) may also pass a newborn hearing-screening program, and later a uSNHL may emerge (Fowler et al., 1999; Fowler et al., 1997; Fowler et al., 2017). A congenital uSNHL may progress into a bilateral SNHL (e.g., due bilateral enlarged vestibular aqueducts (EVAs)) (Johansson et al., 2022). The uSNHL may also be stable or progress in either ear (Lanzieri et al., 2017; Purcell et al., 2017; Ropers et al., 2019). The degree of HL varies from mild to profound uSNHL, where the profound uSNHL is typically referred to as single-sided deafness (SSD).

To find congenital SNHL most NHS programs in Europe use multiple otoacoustic emission (OAE) recordings, in some countries followed by automatic auditory brainstem response (aABR) recordings, and then clinical ABR recordings (Bussé et al., 2021). Most European NHS programs also aim to target uSNHL, although some only focus on identifying bilateral SNHL (Bussé et al., 2021). Given that the PhD project focuses on understanding congenital uSNHL found in the NHS programs, an understanding of both the OAE and the ABR is essential. The OAE is of particular focus in the literature review, as the OAE is the first step in Region Stockholm's universal NHS program which identify congenital uSNHL. Moreover, Study II concentrates on the fundamentals of the OAE.

2.2.1 OAEs

Today, OAEs are recorded worldwide as part of NHS programs due to their usefulness in detecting typical peripheral hearing sensitivity in neonates, objectively and non-invasively (Berninger, 2014; Berninger & Westling, 2011; Kemp, 2002) (Figure 4). Kemp (1978) discovered the OAEs as sounds in the ear canal, based on the travelling wave discovery of von Békésy and Wever (1960), and the theories of the cochlear amplifier by Gold (1948).

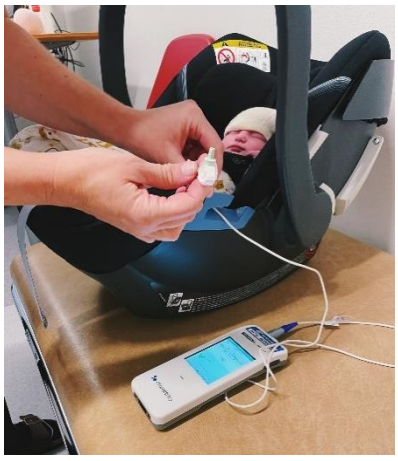

Figure 4. A TEOAE measurement is performed at Uppsala University Hospital on a newborn. A sound is presented in the ear, and a response sound is recorded.

OAEs exists spontaneously (SOAEs) in approximately 65% of human ears, with a prevalence dependent on the recording conditions, and a higher prevalence in females and right ears than in males and left ears (Penner & Zhang, 1997; Talmadge et al., 1993). Different OAEs can also be evoked by different sound stimuli, e.g., a tone (stimulus frequency OAE, SFOAE), a click or transient (transient evoked OAE, TEOAE), or two primary tones where the OAE is spectrally shifted from the stimulus (distortion product OAE, DPOAE).

It has been known since the discovery of the OAEs, that OAEs are caused by processes in the organ of Corti, directly connected with the hearing process (Kemp, 1978). However, the initial theory that all OAEs arise form nonlinear distortion (Kemp, 1978) was questioned when Shera and Guinan (1999) demonstrated results that contradicted a nonlinear theory for low level TEOAEs and SFOAEs. Thus, TEOAEs and SFOAEs are believed to primarily reflect coherent backscattering energy from irregularities in the cochlea, mostly near the peak of the forward travelling wave (Shera & Guinan, 1999; Zweig & Shera, 1995). DPOAEs, on the other hand, are nonlinear events that originate primarily from OHC mechanoelectrical motility (Dallos & Fakler, 2002; Guinan, 2018; Liberman et al., 2002; Ren & He, 2020; Shera & Guinan, 1999). Thus, the distortion (DPOAE) and reflection (TEOAE, SFOAE) emissions appear to some extent to reflect different aspects of cochlear hearing and health (Abdala & Kalluri, 2017).

Another theory of OAE generation that has been dismissed is hemispheric brain specialization based on the OAE stimulus (Sininger & Cone-Wesson, 2004). When the results were replicated, and noise was considered, the theory did not hold (Keefe et al., 2008).

2.2.2 TEOAEs and cochlear processing

Although OAEs have been largely described, our understanding of the overall systems that shape the OAEs is incomplete, especially for the reflection emissions (SFOAEs, TEOAEs), as they are difficult to measure in the small animal models used in auditory research.

The TEOAE screening method used as part of newborn hearing screening in Region Stockholm is fast, non-invasive and offers high intra-individual stability (Franklin et al., 1992; Harris et al., 1991; Johnsen & Elberling, 1982; Marshall & Heller, 1996). In contrast, the inter-individual variability in TEOAE level is large and not fully explored (Berninger, 2007; Bray & Kemp, 1987). Furthermore, it is not known why TEOAEs are larger in right ears and females, as demonstrated in many studies (Aidan et al., 1997; Berninger, 2007; Johansson et al., 2020b; Kei et al., 1997; Thornton et al., 2003), although the effects may not often be

observed in samples smaller than about 500 neonates (Cassidy & Ditty, 2001; Johnsen et al., 1988; Khalfa et al., 1997).

TEOAE levels are largely inherited (Johansson et al., 2020b; McFadden et al., 1996). About three quarters of the TEOAE level was estimated to be inherited based on adult twins with NH (McFadden et al., 1996), whereas we estimate that the TEOAE level at birth may even be close to 100% inherited, based on results from a large group of neonatal twins with NH (Johansson et al., 2020b). The difference in inheritance may be due to androgens during prenatal development, as suggested by McFadden et al. (1996) as the residual was about 25% in their study. In newborns the sex and ear differences in TEOAE level already exist, which could be an argument against this theory. However, children go through several periods of hormone exposures before adulthood (Hines et al., 2015; Lamminmäki et al., 2012), that have shown to influence TEOAEs (Burke et al., 2020).

Many structures and functions of the inner ear are continuously genetically mapped, and based on knock out mice studies, some also have effects on OAEs. Without the protein CEACAM16, coding for Hensen's stripe, situated in the base of two-thirds of the tectorial membrane (Figure 3), TEOAEs and SFOAEs levels are enhanced, and SOAEs are more prevalent (Cheatham et al., 2014). DPOAEs are probably also largely inherited, due to their close relationship to OHC motility and the genetically coded protein prestin (Dallos & Fakler, 2002; Liberman et al., 2002; Ren & He, 2020).

Several recent studies have focused on understanding how the OHC electromotility combines with the forward travelling wave energy, which also has consequences for the understanding of TEOAEs. How different parts of the cochlea work together to process and amplify sound is still unknown, although separate aspects on the cochlear processing and the different mechanisms within the cochlea are known (Guinan, 2022). Recent studies in rodents show results that contradict the theory that OHC regulate the stiff basilar membrane entirely by local feedback (Altoè et al., 2022; Guinan, 2022; He et al., 2018; T. Ren et al., 2016; Tianying Ren et al., 2016). Reticular lamina movement recordings, above the OHC stereocilia (red in Figure 3), show movements that are typically greater than the corresponding basilar membrane movements, in response to acoustic (He et al., 2018; Recio-Spinoso et al., 2017; Ren et al., 2016b) and electric (Ren et al., 2016a) stimulation in the intact cochlea of rodents. The large motility of the reticular lamina has not yet been possible to study in humans, but large movements have been shown in response to stimulation above 5 kHz in mice and gerbils (He et al., 2018; Ren et al., 2016a; Ren et al., 2016b), and below 2 kHz in guinea pigs (Recio-Spinoso et al., 2017). One theory is that the cochlear fluids may play an active role in the interaction between OHCs and the basilar membrane (Tianying Ren et al., 2016). A very recent theory suggests an organ of Corti area pump that starts with OHC vibrations causing cyclic longitudinal fluid motion in the organ of Corti and peri–Deiters-cell tissue. The longitudinal motion changes the

local organ of Corti area, which by reticular-lamina motility drives the scala media fluid that amplifies the traveling wave (Guinan, 2022).

Thus, there may be many causes for a failed TEOAE response at birth, as it reflects irregularities along the travelling wave, which may be affected by outer and middle ear dysfunction, various cochlear micromechanics, (e.g., IHC and OHC), and perhaps also efferent inhibition (Guinan, 2018; McFadden, 1993b).

2.2.3 ABR

The ABR was first described by Sohmer and Feinmesser (1967), and by Jewett et al. (1970) in detail. The ABR is an electrophysiological response measured in the outer ear canal with electrodes placed on the forehead as mastoids (Figure 5). It is most often obtained by using click stimuli (Gelfand, 2016).

Figure 5. An auditory brainstem response (ABR) measurement being prepared by clinical audiologist Maria Drott at Karolinska University Hospital with a 6-month-old infant.

The ABR response consists of up to seven peaks of neural waves that are typically recorded between 0-8 ms after the onset of the click (Gelfand, 2016). Clinically, the first five ABR waves, named ABR I, II, III, IV and V, are typically used to diagnose HL. Many attempts have been made to map the wave peaks to generation sites along the auditory system. However, it seems that the waves after wave II have multiple generators, which makes mapping difficult (Gelfand, 2016). Thus, the ABR waves I-V are usually just referred to as being generated after the cochlea, and along the cochlear nerve path up to the upper brainstem (Eggermont & Don, 1986; Gelfand, 2016). Thus, when studying absolute ABR latencies, e.g., the wave V typically recorded around 5-6 ms in adults, the effects of the middle ear and cochlea are included in the response. By studying the wave I-V interval these effects are excluded from the response, which is used both clinically and in research to study neural transmission times (Eggermont & Don, 1986).

2.3 Etiology of uSNHL

The underlying cause for the auditory dysfunction can be environmental or genetic, where up to 50-60% of the SNHLs in neonates in developed countries have a genetic origin (Morton & Nance, 2006; Smith et al., 2005). However, most of the research on genetic causes is focused on bilateral SNHL. The few study outcomes on uSNHL indicate that the 50-60% is not representable for neonates with uSNHL (Gruber et al., 2017; Liming et al., 2016; Paul et al., 2017; Tropitzsch et al., 2022).

2.3.1 Genetic causes

Existing genetic studies of uSNHL in children, most of which have used a retrospective design, have shown a large variability in the percent of cases (6-43%) that are of genetic origin (Gruber et al., 2017; Haffey et al., 2013; Paul et al., 2017; van Beeck Calkoen et al., 2019). This large spread can be due to selective sampling and the different gene panels used. One study included all children with uSNHL sent to genetic testing, which also poses a risk of selective sampling, although not as apparent as a retrospective design. This study found a genetic cause in only 1 out of 35 children or adults (3%) with congenital uSNHL (Sloan-Heggen et al., 2016). None of the studies of children with uSNHL diagnosed congenital uSNHL based on results from a universal NHS program, as in the PhD thesis study IV, where 28% genetic causes were found (Johansson et al., 2023). Thus, various cases of acquired uSNHL were (presumably) also included in the percentage of non-genetic causes found. One study also included only non-syndromic uSNHL (Gruber et al., 2017).

The most common known genetic cause for uSNHL is autosomal recessive non-syndromic SNHL with a mutation in the GJB2 gene coding for the protein Connexin-26 (Gruber et al., 2017; Haffey et al., 2013; Johansson et al., 2023; Lee et al., 2009; van Beeck Calkoen et al., 2019). However, it is difficult to estimate how common it is due to the variability is study designs with a wide spread in prevalence of 0-31% (Gruber et al., 2017; Haffey et al., 2013; Johansson et al., 2023; Sloan-Heggen et al., 2016; van Beeck Calkoen et al., 2019).

Other known genetic causes for uSNHL include 1) coloboma, heart defect, atresia choanae, retarded growth and development, genital and ear abnormality (CHARGE) syndrome (Haffey et al., 2013; Johansson et al., 2023; van Beeck Calkoen et al., 2019), 2) Pendred syndrome (Johansson et al., 2023; Ropers et al., 2019), and 3) Waardeburg syndrome (Usami et al., 2017; van Beeck Calkoen et al., 2019). A few studies have also found 4) Townes-Brocks (Johansson et al., 2023), 5) VACTERL (Haffey et al., 2013), 6) Goldenhar (Haffey et al., 2013), and 7) Branchiootorenal (BOR) syndrome (Sloan-Heggen et al., 2016) to be causes for uSNHL. Two chromosomal mutations have also been documented in uSNHL; 8) chromosome 8P inverted duplication and deletion syndrome (Johansson et al., 2023), and 9) inversion and deletion in the 13q32–34 region (Paul et al., 2017).

2.3.2 Congenital cytomegalovirus (cCMV) infection

Congenital CMV infection is often referred to as the most common non-genetic cause for SNHL, with a prevalence of about 20-30% (Barbi et al., 2003; Morton & Nance, 2006; Vos et al., 2021). The prevalence for uSNHL has been difficult to estimate due to variability in spread over time, spread across countries, and HL progression in both ears (Fowler et al., 1997; Fowler et al., 2017; Vos et al., 2021). In small groups of children with uSNHL, with risk of selective sampling, the prevalence was around 10% (Paul et al., 2017) to 20% (Arndt et al., 2015; Karltorp et al., 2012). The onset of SNHL may even occur more often after the newborn period, so that many children are not identified through the NHS programs (Fowler et al., 1999; Fowler et al., 2017).

2.3.3 Auditory system malformations

With imaging, malformations obstructing the auditory signal from reaching the auditory cortex may be found. The malformation may be a total blockage of auditory input, e.g., if the cochlear nerve is missing, or it may be a part obstruction, like in EVA where the signal processing in the inner ear is altered to cause mild to profound SNHL (Johansson et al., 2022; Smith et al., 2020). Auditory system malformations are more prevalent in uSNHL compared to bilateral SNHL (Berninger et al., 2022; Johansson et al., 2022; Masuda & Usui, 2019; McClay et al., 2008). The malformation prevalence in uSNHL appear to be similar with computed tomography (CT) of 36-67% (Masuda et al., 2013; Nakano et al., 2013; van Beeck Calkoen et al., 2017), and magnetic resonance imaging (MRI) of 37-64% (Clemmens et al., 2013; Gruber et al., 2017; Johansson et al., 2022; Orzan et al., 2021; Paul et al., 2017; van Beeck Calkoen et al., 2017). The malformation prevalence also appears to be numerically higher (46-67%) when acquired uSNHL is excluded (Johansson et al., 2022; Masuda et al., 2013; Nakano et al., 2013; Orzan et al., 2021).

The absence of a cochlear nerve, as revealed by imaging, usually known as cochlear nerve aplasia or severe hypoplasia, is a common cause for uSNHL. It is also known as cochlear nerve deficiency, although the insufficiency of the nerve is not measured with imaging per se, but can be measured with a combination of imaging and ABR. Cochlear nerve aplasia or severe hypoplasia has been found in 17-50% of children with uSNHL (Clemmens et al., 2013; Gruber et al., 2017; Johansson et al., 2022; Orzan et al., 2021; Paul et al., 2017; van Beeck Calkoen et al., 2017). The large spread in prevalence may be explained by selective sampling, MRI resolution, and/or the rate of congenital or acquired uSNHL included in the studies.

Another common malformation cause for uSNHL is inner ear malformations, with a prevalence of 28-46% (Clemmens et al., 2013; Orzan et al., 2021; Paul et al., 2017; van Beeck Calkoen et al., 2017). Some malformations in uSNHL have a genetic cause. EVA in the inner ear is associated with several syndromes including Pendred, Townes-Brocks, BOR, CHARGE, and Waardenburg syndromes (Johansson et al., 2022; Pryor et al., 2005). EVA

may be both bilateral or unilateral in uSNHL and has a prevalence around 8-25% in children with uSNHL (Clemmens et al., 2013; Johansson et al., 2022; Orzan et al., 2021; Paul et al., 2017; van Beeck Calkoen et al., 2017). Bilateral EVAs have a large risk of deterioration of hearing in both ears (Jackler & De La Cruz, 1989).

2.4 Consequences of uSNHL

Children with uSNHL have a larger risk of speech-language delays, psychosocial challenges and academic difficulties compared to children with NH (e.g., reviews by Kuppler et al. (2013); Lieu (2013); McKay et al. (2008)).

Furthermore, children with uSNHL generally have worse sound source localization (Bess & Tharpe, 1984; Bess et al., 1986; Humes et al., 1980; Johansson et al., 2020a; Newton, 1983), and understanding of speech when noise or competing speech is present compared to children with NH (Bess et al., 1986; Bovo et al., 1988; Johansson et al., 2020a; Ruscetta et al., 2005).

Children with uSNHL may also experience listening fatigue (Bess et al., 2020), and other effects on cognition (Ead et al., 2013). Lower IQ has also been reported (Purcell et al., 2016), although it is yet unknown how much of the lower IQ can be attributed to syndromes, and non-hearing-related factors.

Untreated uSNHL may also lead to an aural preference syndrome (Gordon et al., 2015; Kral et al., 2013b), neural reorganization (Kral et al., 2013a; Kral et al., 2013b), and/or auditory deprivation (Zhang et al., 2016). The aural preference has been documented in both some animal models, and children that have received sequential cochlear implants (CIs) (Gordon et al., 2015). It occurs when the auditory input from a normal hearing ear (NE) gets overrepresented in the auditory cortex, whereas eventually the central representations become weaker from the impaired ear (IE) (Gordon et al., 2015).

2.4.1 Changes in neural transmission and brain circuits

During early sensitive periods it is crucial that adequate environmental input is received in order for a function or skill to develop normally (Knudsen, 2004). For children with congenital uSNHL the auditory signal on the impaired side may be insufficient for some functions, e.g., sound localization, to develop typically.

Longer periods of uHL have shown to change neural circuits in the brain (Gordon et al., 2013; Jiwani et al., 2016; Kral et al., 2013a; Kral et al., 2013b; Tillein et al., 2016). Changes have been demonstrated after a mild to severe uHL was induced near birth (Kral et al., 2013b; Polley et al., 2013; Popescu & Polley, 2010) and for profound congenital uSNHL in lab animals (Kral et al., 2013a; Kral et al., 2013b; Tillein et al., 2016). In children with CIs, changes in neural circuits have been demonstrated for bilaterally deaf children who experienced a long duration of time between their first and second CI (Gordon et al., 2013; Jiwani et al.,

2016). Most noteworthy, a recent study showed that changes to neural circuits resolved with persistent CI use in a few children with SSD with cCMV infection etiology (Polonenko et al., 2017a). The changes in neural brain patterns over time is a factor to take into consideration in the intervention of children with uSNHL (Gordon et al., 2015; Purcell et al., 2020).

2.4.2 Speech-language delays and auditory behavior in uSNHL

Based on the many reviews of the situation for children with uHL there is consensus in that children with uHL are at risk for speech-language delays (Anne et al., 2017; Gordon et al., 2015; Huttunen et al., 2019; Kuppler et al., 2013; Lieu, 2004; Lieu, 2013; McKay et al., 2008; Tharpe, 2008). However, almost all reviews, despite having somewhat different aims, address the problem with few studies of high quality, small samples, and heterogeneity in outcome measures (Anne et al., 2017; Huttunen et al., 2019; Kuppler et al., 2013).

2.4.2.1 Early speech-language delays and early auditory behavior

Only a few studies have investigated the communication development for infants and young children with uSNHL, from infancy to preschool years (Borg et al., 2007; Borg et al., 2002; Fitzpatrick et al., 2019; Kishon-Rabin et al., 2015). Although mixed outcomes, auditory behavior were often delayed, as well as some speech and language abilities (Borg et al., 2007; Borg et al., 2002; Fitzpatrick et al., 2019; Kishon-Rabin et al., 2015). All studies analyzed uSNHL, mixed uHL and conductive uHL combined, and no distinction between congenital and acquired uHL was made in the analysis (Borg et al., 2007; Borg et al., 2002; Fitzpatrick et al., 2019; Kishon-Rabin et al., 2015).

Specifically, 21% of infants with uHL showed delayed auditory behavior (median age: 9 months, n = 34, 56% SNHL) (Kishon-Rabin et al., 2015). The results also indicated that risk factors for developmental delay (e.g., > 48 hours in intensive care unit, obvious syndromes, CMV) did not seem to be the only reason for the delay. Delay in auditory behavior was still four times more common in uHL compared to in children with NH after adjustment for the risk factors ($p = 0.01$) (Kishon-Rabin et al., 2015). Auditory behavior was assessed with the Infant-Toddler Meaningful Auditory Integration Scale (IT-MAIS) questionnaire (Kishon-Rabin et al., 2015). However, with such a young group of children, without any genetic testing results, it is difficult to rule out syndromic factors contributing to the delay.

Similarly, preschoolers with uHL has been shown to fall behind in auditory behavior compared to children with NH (median age: 48 months, n = 38, 92% reported congenital, 63% SNHL) (Fitzpatrick et al., 2019). This was assessed with the Parents' Evaluation of Aural/Oral Performance of Children (PEACH), as well as the Children's Home Inventory for Listening Difficulties (CHILD) (Fitzpatrick et al., 2019). PEACH only showed statistical

significance for noisy environment, not for quiet ones, whereas CHILD showed overall delay in hearing behavior (Fitzpatrick et al., 2019).

Preverbal vocalization may be delayed already during the first year of life (Kishon-Rabin et al., 2015). Preverbal vocalization was delayed in 41% of infants with uHL, and delays were nine times more common for uHL compared to children with NH, after the adjustment for developmental risk factors ($p < 0.0001$) (Kishon-Rabin et al., 2015). This was assessed with the Production of Infants Scale Evaluation (PRISE) questionnaire.

Preschoolers with uHL also show delays in expressive and receptive language, assessed with the Preschool Language Scale (PLS-5) (Fitzpatrick et al., 2019). However, receptive vocabulary and speech perception was similar to that of NH controls, assessed with the Goldman-Fristoe Test of Articulation Sound-in Words subtest (GFTA-2) and the Peabody Picture Vocabulary Test (PPVT-4) (Fitzpatrick et al., 2019). Similarly, a language test with nine subtests for preschoolers with uSNHL in Sweden was evaluated by Borg et al. (2002). It was reported that 4-year-olds (n = 6) and 5-year-olds (n = 15) were delayed in language development, whereas no significant deviation from children with NH could be found for 6-year-olds (n = 20). Nonetheless, when the tests were broken down into subtests, and the whole group with uSNHL (n = 41) was evaluated, no significant differences were reported (Borg et al., 2007).

2.4.2.2 Later speech-language delays and auditory behavior

In one study it was found that 74 children with uHL demonstrated worse oral language scores than did their 74 siblings with normal hearing (Lieu et al., 2010). In study I we found that aided and unaided auditory behavior were delayed in 10-11-year-old children with congenital uSNHL (Johansson et al., 2020a), as measured with PEACH, i.e., the same questionnaire and almost the same result as for the preschoolers (Fitzpatrick et al., 2019). Furthermore, the abbreviated profile of HA benefit (APHAB) showed more frequent problems with communication in quiet, noise and reverberation compared to children with NH (Johansson et al., 2020a).

Two longitudinal studies demonstrate that with awareness of speech-language delays, speech-language abilities in uHL (Lieu et al., 2012) and reported speech difficulties for severe uHL (Peckham & Sheridan, 1976) can to some extent be resolved. For example, Lieu et al. (2012) found that the oral language previously delayed (Lieu et al., 2010), improved over time. However, no improvement in school performance could be found with various outcome measures.

2.4.3 Academic difficulties

In the 1980s several studies investigated school outcomes for children with uHL in the USA, and found that grade repetition was up to 10 times more common in children with uHL compared to all children in the same elementary school region (Bess & Tharpe, 1986;

Bovo et al., 1988; Klee & Davis-Dansky, 1986). In the study by Bess and Tharpe (1986) 35% of the children with uHL had repeated a grade (n = 21 out of 60), as compared to 3.5% for all children in the mid-Tennessee region (Tharpe, 2008).

In a group of children with uSNHL in Denmark the grade repetition was 7% (n = 2 out of 30) (Hartvig Jensen et al., 1989), which also appear to be a high number, as the number of students that had repeated a grade in Denmark in 1988 (up to grade 8) was about 0.6 % (personal communication with Hans Henrik Sievertsen, University of Bristol, based on data from: Landersø et al. (2017)). However, it should be noted that the children that dropped out of school were not included in the 0.6%, so this figure may be somewhat higher, but not as high as 7%.

The academic and speech-language difficulties may also be more common if the HL is in right ears, although the evidence in weak. In Denmark, the two cases of grade repetition were in right ears, so the grade repetition was in effect 18% (n = 2 out of 11) in right ear uHL and 0% (n = 0 out of 19) in left ear uHL. In a subgroup of 8 out of 25 children that repeated a grade five children had right ear uHL, wheres three had left ear uHL.

In recent years school performance has also been shown to be affected by uHL. For example, Lieu et al. (2010) showed that children with uHL were four times more likely to have individual education plans, and three times more likely to need speech-language therapy than their siblings with NH. Furthermore, around 50% of the children with uHL still had an individual education plan at follow-up three years later, and approximately 20% still needed speech-language therapy (Lieu et al., 2012).

2.4.4 Effects on psychosocial behavior, cognition, listening fatigue and IQ

Children with uHL also have larger risk of psychosocial challenges compared to children with NH. Excessive behavior including aggression and social withdrawal was reported in 42% of children with uHL by Stein (1983), despite adequate school performance. Similarly, feelings of embarrassment, annoyance, confusion and helplessness was reported to be common among children with uHL (Giolas & Wark, 1967). A recent study of school aged children with minimal and mild HL (15-40 dB HL) found that auditory and cognitive tasks were equally impaired for children with asymmetric HL (including uHL) and symmetric HL (Moore et al., 2019).

A recent study found that listening fatigue, measured with questionnaires, was impaired to the same degree in uHL and bilateral HL (Bess et al., 2020).

Lower IQ scores also have been reported for children with uHL compared to children with NH, as indicated by a meta-analysis (Purcell et al., 2016) and most of the included studies (Klee & Davis-Dansky, 1986; Lieu et al., 2013; Martínez-Cruz et al., 2009; Schmithorst et al., 2014). However, it is yet unknown how much of the lower IQ can be attributed to syndromes, and specifically non-hearing-related factors.

School aged-children with SSD may have reduced accuracy and efficiency associated with phonological processing (Ead et al., 2013). They may also have an impaired executive control function when engaged in maintaining verbal information in the face of processing incoming, irrelevant verbal information compared to siblings (Ead et al., 2013).

2.5 Auditory stimulation to ameliorate the consequences of uSNHL

Habilitation of children with uSNHL has for a long time been a neglected research field (Bess & Tharpe, 1984; Lieu, 2004), with many review studies requesting more research (Appachi et al., 2017; McKay et al., 2008; Tharpe, 2008), and few outcome studies.

The biggest challenge when habilitating uSNHL is perhaps the limited amount of evidence from successful treatment options. A "one size fits all" approach does not seem to apply to uHL (Appachi et al., 2017; Johansson et al., 2020a; McKay et al., 2008; Tharpe, 2008), which is why studies need to be focused on more homogenous groups of uHL or include larger samples in which results are divided by relevant factors (conductive uHL vs uSNHL, conductive uHL vs acquired uHL, even degree of uHL).

Another challenge with aiding uSNHL is that some children with uSNHL will have trouble in school, and others will perform satisfactorily. Research describing predictors of who will struggle and who will not, e.g., based on etiology is lacking (McKay, 2010; Tharpe, 2008). Since perception of benefit of a HA typically is correlated with HA use (e.g., Muños et al, 2015) the motivation for early amplification is a challenge.

The intervention options that aim at restoring binaural hearing to children with uSNHL are HAs and CIs. Bone-anchored hearing devises and contralateral routing of the signal (CROS) devices may also be of benefit for uSNHL (Appachi et al., 2017), but they do not restore binaural hearing, as they work by transfering the signal to the NH ear through scull vibrations and acoustic transmission, respectively. Remote microphone systems (previously known as FM-systems) may also be of help in uSNHL (Updike, 1994), but also focus on transferring the signal to the NE, not restoring hearing. The focus of this thesis is on amplification in the impaired ear, so CROS, bone-anchored devices, and remote microphone systems will not be covered here.

Several outcome studies for children with SSD have been published during the last decade (Arndt et al., 2015; Arras et al., 2022; Beck et al., 2017; Benchetrit et al., 2021; Deep et al., 2020; Ehrmann-Mueller et al., 2020; Ganek et al., 2020; Hassepass et al., 2013; Plontke et al., 2013; Polonenko et al., 2017a; Polonenko et al., 2017b; Tavora-Vieira & Rajan, 2015, 2016; Thomas et al., 2017). A CI can provide improvements on several aspects of communication and learning for children with SSD, especially if the child with congenital SSD is fitted before 4 years of age (Benchetrit et al., 2021; Polonenko et al., 2017a; Thomas et al., 2017). However, more research is still needed to predict which children with SSD that will benefit most from the intervention, as longitudinal outcomes are still few (Arras et al.,

2022; Benchetrit et al., 2021). The initial plan was to also include CI for SSD in the thesis, but the thesis became too comprehensive for it to be included. This thesis will cover HA intervention.

2.5.1 HA amplification outcomes

Results of HA intervention outcomes have shown to be heterogeneous in children with uSNHL (Benchetrit et al., 2022; Briggs et al., 2011; Johansson et al., 2020a; Johnstone et al., 2010; Rohlfs et al., 2017; Updike, 1994). To my knowledge no HA outcomes have been demonstrated for children fitted with HAs early, before 4 years of age. Nonetheless, review studies of uHL and asymmetric SNHL still find recommendations for early HA intervention based on the risk of deprivation and aural preference without or with delayed intervention (Gordon et al., 2015; Purcell et al., 2020). According to American Academy of Audiology's pediatric guidelines children with aidable uHL "should be considered candidates for amplification in the impaired ear due to evidence for potential developmental and academic delays" (AAA, 2013).

The most positive HA outcomes for children with uHL come from questionnaire results (Benchetrit et al., 2022; Briggs et al., 2011; Johansson et al., 2020a). Two studies were based on HA trials, with different setups (Benchetrit et al., 2022; Briggs et al., 2011), and Study I evaluated HA outcomes in children that had used HAs for 1.5-4 years (Johansson et al., 2020a). The two HA trials included children with conductive, mixed and sensorineural uHL (Benchetrit et al., 2022; Briggs et al., 2011), where most subjects included by Benchetrit et al. (2022) had conductive uHL (59%) and all children also used a FM-system in school. Johansson et al. (2020a) included only children with congenital uSNHL and used the PEACH questionnaire for the parents and APHAB for the children. Both HA trials also used the same five questionnaires: CHILD parent and child questionnaires and the Learning Inventory For Education (LIFE) student and teacher questionnaires, as well as the Hearing Environments and Reflection on Quality of Life (HEAR-QL) questionnaire for the child.

The two HA trials reported general HA-benefit in home and school by parent, child and teacher, and quality of life based on the questionnaire results. Benchetrit et al. (2021) only reported overall five-questionnaire benefit, by using mean averaging of all survey's standardized scores (although scores for each questionnaire could be found as online material, but no aided vs unaided score was compared per questionnaire). Similarly, children with congenital uSNHL showed HA benefit in one-to-one communication (Johansson et al., 2020a) with APHAB, although the children experienced neither benefit nor dis-benefit in noisy and reverberant listening situations (Johansson et al., 2020a). Differences could be attributed to the type of uHL (congenital/acquired, conductive/sensorineural/mixed), or the differences in study designs. Benchetrit et al. (2022) included the largest study group and used a cross-over design with follow-up measurements and considered acclimatization effect, although a lot of missing data

existed in the follow-up sessions. HA trials using subjective measurements also have large risks of placebo effects (Dawes et al., 2013; Dawes et al., 2011), that did not seem to be controlled for in either HA trial (Benchetrit et al., 2022; Briggs et al., 2011). Dawes et al. (2013) found that if a HA was named "new", it was rated higher on sound quality, showed higher speech in noise performance, and subjects also preferred the "new" HA, although it was acoustically identical to the HA named "conventional".

Neither HA benefit, nor disbenefit has been demonstrated for speech recognition in quiet, noise and competing speech in the sound field (Briggs et al., 2011; Johansson et al., 2020a; Updike, 1994).

Disbenefit in sound localization has been demonstrated for older children with uSNHL (10-11-year-olds, Johansson et al. (2020a); 10-14-year-olds, (Johnstone et al., 2010)) fitted with HAs late in development (at 5-12 years of age). This may be explained by HAs not preserving the binaural cues needed for accurate SLA, as has been shown for bilateral HAs for bilateral HL (Van den Bogaert et al., 2006). It is therefore noteworthy that HA benefit in sound localization was shown for the younger children with USNHL fitted earlier in development (at the age of 4-6) by Johnstone et al. (2010), indicating that plasticity may overcome altered binaural cues. It should be noted that the younger children with earlier HA fittings and benefit still performed worse in aided sound localization compared to the older children with later HA fittings and disbenefit (Johnstone et al., 2010), so the plasticity hypothesis need further study, and longitudinal follow-up to better handle development effects.

3 Research aims

The overall aim of this Ph.D. thesis was to study the causes and mechanisms behind very early diagnosed congenital uSNHL, and the effect of HA amplification on the hearing development, speech-language development, and the maturation of the auditory pathways.

More specifically the aims were to:

- Evaluate the effect of HA amplification in children with congenital uSNHL, using subjective and objective tests targeting everyday life listening (Study I)
- Estimate TEOAE heritability in newborns and describe genetic and non-genetic contributions, as the TEOAE is a basis for universal NHS programs worldwide, and therefore a first step in diagnosting congenital uSNHL (Study II)
- Describe congenital uSNHL, genetic and non-genetic causes, malformations, auditory profiles, and the affected auditory mechanisms (Study III and IV)

4 Materials and methods

4.1 Study design

Four studies are included in the thesis, with various subject groups, test batteries, analysis methods and study designs (Table 1).

Table 1. Method overview for each study.

	Study I	**Study II**	**Study III**	**Study IV**
Design	Observational study with within-subjects repeated measures	Prospective cross-sectional data collection with retrospective analysis of the subset of twins	Prospective cross-sectional, part of longitudinal project	Prospective cross-sectional, part of longitudinal project
Subject age	10-11 years of age	Neonates	Infants	Infants
Subject group size	N = 6 children with congenital uSNHL	N = 454 NH twins, N = 21 199 NH non-twins	N = 20 infants with congenital uSNHL	N = 20 infants with congenital uSNHL
Test battery	ABR, Aided SII, APHAB and PEACH surveys, ARTs, Bekesy audiometry, HA datalogging, Otomicroscopy, sound localization test, SRT in competing speech, tympanometry	TEOAE	ABR, ARTs, cCMV infection test, DPOAE, MRI, otomicroscopy, TEOAE, Tympanometry	Genetic testing analyzed together with results from Study III
Analyses	Descriptive analysis, nonparametric and parametric tests, regression analysis	Descriptive analysis, nonparametric and parametric tests, correlations, effect sizes, heredity model with boot strapping	Descriptive analysis, nonparametric tests, linear regression, linear mixed modeling	Descriptive analysis, parametric proportion difference test

ABR = auditory brainstem response; ART = Acoustic stapedius reflex threshold, APHAB = The Abbreviated Profile of Hearing Aid Benefit; cCMV = congenital cytomegalovirus; DPOAE = Distortion product otoacoustic emission; HA = hearing aid; MRI = magnetic resonance imaging; NH = normal hearing; PEACH = The parents evaluation of aural/oral performance of children; uSNHL = unilateral sensorineural hearing loss; SRT = Speech recognition threshold; SII = speech intelligibility index, TEOAE = Transient-evoked otoacoustic emission

4.1.1 Study design Study I

The subjects were invited to a research visit lasting approximately 3 hours. The test battery included measurements of pure tone thresholds (PTTs), the ABR, SLA, speech recognition thresholds (SRTs) in competing speech, Aided speech intelligibility index (SII), HA datalogging, tympanometry and acoustic reflex thresholds (ARTs). Otomicroscopy was performed by an experienced otologist. The Abbreviated Profile of Hearing Aid Benefit (APHAB) was filled in by the child and the Parents evaluation of aural/oral performance of children (PEACH) was filled in by the accompanying parent.

Within-subjects repeated measures were used to study the effect of the HA in tests simulating everyday life situations. The subject's own HA was used in the measurements of SRTs in competing speech and SLA, and the questionnaires (PEACH and APHAB) were answered with an aided and unaided condition in mind.

Left/right ear and unaided/aided start conditions were randomized.

4.1.2 Study design Study II

Study II used a prospective cross-sectional data collection that was ongoing from November 1998 to the end of 2004 when the universal NHS was first introduced in Region Stockholm, starting at Karolinska University Hospital, Huddinge, and Södertälje Hospital, Södertälje, Sweden. During the 6-year period >30 000 newborns were screened with 98% coverage rate (Berninger, 2007, 2014; Berninger & Westling, 2011). A retrospective analysis of the TEOAEs of a subset of twins, focused on within and between twin pair analysis was included in Study II (Johansson et al., 2020b). The TEOAEs of twins were also compared to non-twins for ear and sex differences.

Intra-twin pair relationships were studied to estimate heredity. The intra-twin pair correlations for twin pairs of same-sex (SS) and opposite-sex (OS) were compared, as well as estimated monozygotic (MZ) and dizygotic (DZ) relationships. Falconer's formula was used to estimate broad heritability based on the difference between estimated MZ and DZ correlations (Falconer & Mackay, 1996). The TEOAE correlations of twins paired randomly was used as a comparison to biological-twin-pair correlations.

A mathematical model was used to estimate a MZ within-twin pair correlation coefficient and compare the correlation coefficient to that of the estimated DZ twin correlation coefficient.

The OS twin pairs are DZ. Hence, the TEOAE variance and correlation coefficient for the DZ twins was calculated from the OS twin pairs.

According to Weinberg's differential rule (Fellman & Eriksson, 2006), the rate of DZ twinning is twice the rate of twin maternities in which the twins are of OS (i.e., $N_{DZ} = 2N_{OS}$, our set: $n_{DZ\ estimated} = 2n_{OS} = 152$). The MZ twinning rate is the difference between the rates of SS and OS twin pairs ($N_{MZ} = N_{SS} - N_{OS}$, our set: $n_{MZ\ estimated} = n_{SS} - n_{OS} = 151-76 = 75$). Accordingly, the number of DZ pairs in the SS set should then approximately be equal to the estimated number of MZ twin pairs (our set: $n_{DZ\ estimated\ in\ SS\ group} = n_{SS} - n_{MZ\ estimated} = 151-75 = 76 \approx n_{MZ\ estimated}$). Additionally, based on the 50% probability of a MZ twin set (75/151 = 0.50), and 50% probability of a DZ twin set (76/151 = 0.50), for a random twin pair in the SS twin sets, MZ twin's TEOAE variance was estimated as:

$$\sigma_{SS}^2 = P(MZ)\sigma_{MZ}^2 + P(DZ)\sigma_{DZ}^2 = \frac{1}{2}(\sigma_{MZ}^2 + \sigma_{DZ}^2) \Rightarrow$$

$$\sigma_{MZ}^2 = 2\sigma_{SS}^2 - \sigma_{DZ}^2, \quad (1)$$

where *P* is probability, and σ_{SS} are estimated from the TEOAE levels of the SS set, and σ_{DZ} from the TEOAE levels of the OS set.

Then, the correlation coefficient (ρ_{ss}) was calculated according to:

$$\rho_{SS} = \frac{\sum_{i=1}^{n}(X_i - \bar{X})(Y_i - \bar{Y})}{\sqrt{\sum_{i=1}^{n}(X_i - \bar{X})^2 \sum_{i=1}^{n}(Y_i - \bar{Y})^2}}$$

$$= \frac{P(MZ)\rho_{MZ}\sigma_{XMZ}\sigma_{YMZ} + P(DZ)\rho_{DZ}\sigma_{XDZ}\sigma_{YDZ}}{\sigma_{XSS}\,\sigma_{YSS}}, \quad (2)$$

where *P* is probability, and *X* is for twin 1 and *Y* for twin 2 in each twin set. It is assumed that the standard deviation is equal for twin 1 (x) and twin 2 (y) in the population. Thus:

$$\rho_{SS}\sigma_{SS}^2 = \frac{1}{2}(\rho_{MZ}\sigma_{MZ}^2 + \rho_{DZ}\sigma_{DZ}^2) \quad (3)$$

From equations 1 and 3 the correlation coefficient for the MZ twins is estimated as:

$$\rho_{MZ} = \frac{2\rho_{SS}\sigma_{SS}^2 - \rho_{DZ}\sigma_{DZ}^2}{2\sigma_{SS}^2 - \sigma_{DZ}^2}, \quad (4)$$

where the variance for the SS twins was estimated as 0.5(variance for twin 1 + variance for twin 2) from the SS set, and the variance for the DZ twins was estimated as 0.5(variance for twin 1 + variance for twin 2) from the OS set.

Falconer's formula was then used to calculate broad heritability (Falconer & Mackay, 1996):

$$H^2 = 2(r_{MZ} - r_{DZ}), \quad (5)$$

where the MZ and DZ intra-twin pair correlation coefficients (r_{MZ} and r_{DZ}) are estimated from equations 1-4. Falconer's formula is twice the difference in correlation between MZ and DZ twins. The formula originates from MZ twins sharing all the same genes, whereas DZ twins normally share half their genes. Thus, MZ twins are on average twice as genetically similar as DZ twins (Falconer & Mackay, 1996).

4.1.3 Study design Study III and IV

The subjects were invited to two research visits (Study III and Study IV).

The first visit took 2.5-3 hours and included audiologic measurements, i.e., ABRs, ARTs, DPOAEs, TEOAEs, and tympanograms (Study III). An experienced otologist performed otomicroscopy and gained a medical history from the infant and parents, including family history of HL, and prenatal and perinatal history. A CMV infection test on the mother was also ordered and taken as soon as possible (Study III).

The second visit included MRI of the inner ears and cochlear nerves (Study III), as well as a blood test for genetic testing (Study IV). A few subjects took the blood test on another occasion instead, typically in combination with a research follow-up visit in the longitudinal part of the research project (longitudinal results not presented in thesis).

4.2 Subjects

Study I, III and IV recruited children with congenital uSNHL, while study II recruited neonatal twins and non-twins with NH.

4.2.1 Subjects Study I

4.2.1.1 Inclusion criteria

(1) Children with non-syndromic congenital uSNHL aged 6 to 11 years of age, (2) pure tone thresholds (PTTs) of ≤20 dB HL (0.25-8 kHz) in the NE, (3) Pure tone average (PTA, 0.5, 1,

2 and 4 kHz) of >30 dB HL and <90 dB HL in the IE, and (4) at least 6 months of HA use in the impaired ear.

4.2.1.2 Exclusion criteria

Not hearing-screened at birth in Stockholm Regional Council.

4.2.1.3 Eligible and recruited subjects

Subjects were identified in Karolinska University Hospital's hearing habilitation database of children and youth with HL. Seven children were eligible according to the inclusion criteria.

Six subjects agreed to participate. One eligible subject declined the invitation to the study. The participants were 9.7-10.8 years of age at the research visit (50% males, 50% right IEs). The participants had 1.5-5.8 years of HA experience (time since they were first fitted with a HA). The age of first HA fitting was after 4.8 years of age for all subjects, considerably late in speech-language and hearing development.

All subjects had a TEOAE pass in their NEs and several TEOAE non-passes in their IEs (Region Stockholm NHS program details: (Berninger, 2007, 2014; Berninger & Westling, 2011)). The HLs were categorized as uSNHL in the medical records, which was confirmed by the study of recent audiograms.

4.2.2 Subjects Study II

4.2.2.1 Inclusion criteria

All twin pairs that passed TEOAE NHS in all four ears at the same test occasion, and had valid data on the mother's name, ear tested, sex, and test date. All non-twins with valid data on the mother's name, ear tested, sex, and test date.

4.2.2.2 Eligible and recruited subjects

In a clinical database 642 twins were identified as having passed NHS (i.e., TEOAE) at Karolinska University Hospital, Huddinge, and Södertälje Hospital, Södertälje, out of >30 000 screened newborns during the recruitment period of 6 years. A costum-made Matlab program was used to identify twins that passed the strict inclusion criteria regarding valid data.

The TEOAEs of 454 twins in 227 pairs were eligible for the study and were extracted from the TEOAE equipment for offline analysis including the relevant TEOAE data (TEOAE levels, TEOAE reproducibility, signal-to-noise ratio (SNR) in different frequency bands, number of measured sweeps), the data on mother's name (coded before analysis), ear tested, sex, and test date. Of the 227 twin pairs, 151 were of SS (66.5%) and 76 of OS (33.5%). Of all twins 51.1% were male, and 51.6% of the SS twin pairs were male.

The non-twin comparison group for sex and ear differences consisted of the TEOAEs of 21 199 newborns (50.3% males).

4.2.3 Subjects Study III-IV

4.2.3.1 Inclusion criteria

(1) One ear failing and one ear passing TEOAE universal NHS, (2) An ABR click threshold (ABRthr) of >30 dB nHL in the IE, and (3) An ABRthr of ≤20 dB nHL in the NE.

TEOAE pass criteria comprised: ≥70% whole wave reproducibility, SNR ≥4 dB in at least three of the upper four wide-frequency bands provided by the TEOAE instrument, and ≥50 sweeps.

4.2.3.2 Exclusion criteria

Bilateral HL, mixed HL and unilateral conductive HL.

4.2.3.3 Eligible and recruited subjects

The recruitment of subjects from the NHS program was divided into several steps to invite all infants with congenital uSNHL and exclude all infants with temporary uHL, e.g., due to otitis media with effusion.

The doctoral student was notified by the NHS staff at Karolinska University Hospital, Huddinge with a medical record notification when an infant was identified with possible uHL. The definition for possible uHL was several TEOAE non-passes in an IE with TEOAE pass in a NE and passed automatic ABR screening in the NE (≤30 dB nHL), while showing a non-pass in the IE (>30 dB nHL). At the first audiologic test visit following the NHS (typically including measurements of the ABR, the auditory steady-state response, and tympanograms) the family was informed of the study if ABRthr of >30 dB nHL was measured in the IE, and ≤25 dB nHL ABRthr in the NE. If the subjects showed ABRthrs of ≤20 dB nHL in the NE and >30 dB nHL in the IE at the first research visit in the study, they were included as participants. If the subject showed signs of otitis media with effusion at the research visit that explained elevated ABRthrs, but uSNHL may still exist as well (mixed HL), they were rescheduled for another visit after about 4-6 weeks.

Sixty-eight potential subjects were identified in the universal NHS based on TEOAE non-passes in an IE and TEOAE pass in a NE, as well as automatic ABR in the NE of ≤30 dB nHL, and non-pass in the IE of >30 dB nHL.

Twenty subjects were invited to the study after exclusions at the first clinical audiologic measurements visit, and first research visit (see Figure 6 for estimated number and included number of participants). Exclusions were typically made due to temporary otitis media with effusion (OME) that fully explained the elevated ABRthrs in the IE, and ABRthr

of ≤30 dB nHL in the IE possibly due to previous OME, or bilateral HL. All 20 infants with congenital uSNHL and their parents agreed to participate in the study.

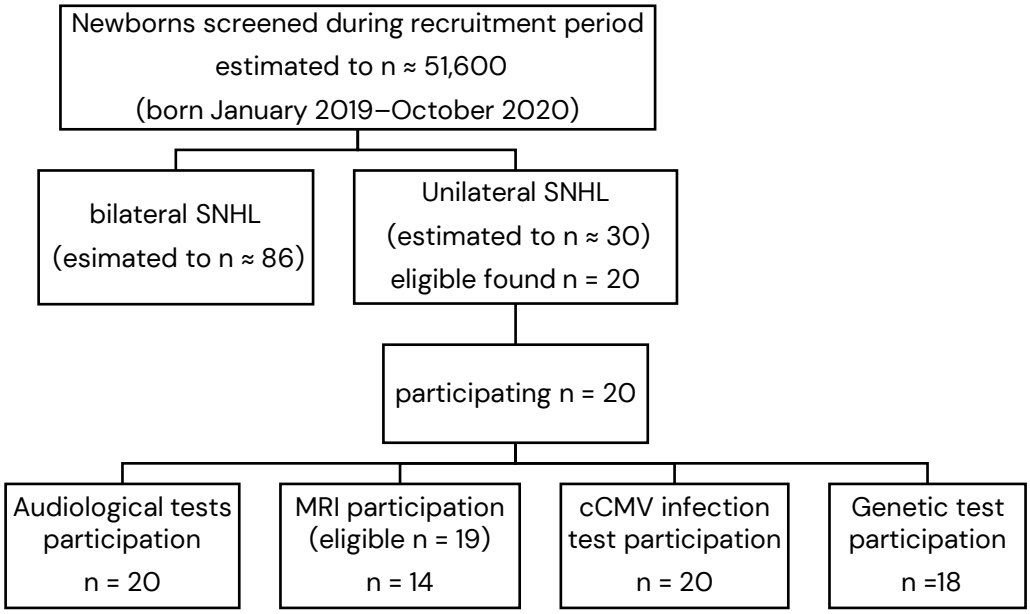

Figure 6. The estimated and included number of participants in study III and IV. Estimated participants are determined from previous Region Stockholm newborn hearing screening (NHS) prevalence of sensorineural hearing loss (SNHL) (Berninger & Westling, 2011), and newborns 2019-2020 in Region Stockholm (Statistics Sweden, 2022). cCMV = congenital cytomegalovirus; MRI = magnetic resonance imaging. Adapted version of Figure 1 by (Johansson et al., 2022) including genetic testing from Johansson et al. (2023). Published by MDPI.

4.3 Materials and procedure

A wide range of measurements were used in studies I-IV. Tympanometry, ARTs, ABRs, and TEOAEs were measured in several studies (see test battery in Table 1).

4.3.1 Tympanometry and ARTs (Study I and III)

Bilateral otomicroscopic examination, bilateral tympanometry and ipsilateral ARTs in the impaired ear were performed in study I and III to ensure normal middle ear conditions and study the reflex arc. In study I a Madsen Otoflex 100 tympanometer (GN Otometrics, Denmark) was used with a probe tone at 226 Hz (ipsilateral stimulation at 1 kHz up to ≤100 dB HL). In study III a GSI Tympstar tympanometer (Grason-Stadler, Eden Prairie, MN, USA) was used with a stimulus frequency of 1000 Hz, using an ascending method in steps of 5 dB with a maximum of 105 dB HL (as recorded in a 2-cc coupler).

4.3.2 ABR (Study I and III)

ABRs were recorded monaurally in both ears using rarefaction clicks (100 µs). Insert earphones (EAR Tone, Etymotic Research Inc, USA) presented the clicks at 39.1 Hz repetition rate with Eclipse EP25 (program version 4.3, Interacoustics, Denmark).

In both studies 70 dB nHL was the ABR stimulus level measured first, raised up to a maximum of 90 dB nHL in the IE if waves I and V could not be discerned at lower input levels, and up to 80 dB nHL in the NE. The stimulus was then decreased, in 10 dB steps whenever possible, down to the ABRthr or 20 dB nHL.

The response quality was enhanced by using up to 10 000 sweeps at stimulus levels close to the ABRthr, and ≈2 000 sweeps for the other stimulus levels.

Electrodes (Ag/AgCl) were put on the forehead, the vertex electrode on the top of the forehead just below the hairline, and the ground a few centimeters lower and to the side. Two electrodes were also placed on the left and right mastoids. The subjects were placed in a supine position, in study I in a comfortable chair, and in study III in the parent's arms or in a baby carrier. Lights in the audiometric test room were turned off during the ABR recordings to minimize electric interferences.

If no ABR-waves could be determined the wave reproducibility (ρ) was objectively defined in the time domain (1-15 ms) for the entire ABR, and the lack of a response was confirmed if ρ was <70% (ρ = 70% corresponds to SNR = 3.7 dB (Berninger et al., 2014)). The wave I of each ABR recording was objectively confirmed by a ρ ≥70% within a time window of 1-1.5 ms encompassing the wave. The ABR wave V at threshold was confirmed with a ρ≥70% within a time window of 1-1.5 ms encompassing the wave. The amplitudes for the ABR waves were quantified based on the difference between the vertex positive peak maximum and succeeding minimum (Berninger et al., 2014).

Contralateral masking was applied when needed (Sklare & Denenberg, 1987).

4.3.3 Pure-tone thresholds (PTTs, Study I)

PTTs were measured monaurally via insert ear phones (EAR Tone; Etymotic Research Inc, USA) in both ears using a computerized fixed-frequency Bekesy technique (at 0.125, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, and 8 kHz). The Bekesy technique is characterized by high reliability (Erlandsson et al., 1979; Paintaud et al., 1994). Contralateral masking was applied when needed (Sklare & Denenberg, 1987).

4.3.4 HA acoustic gain, and speech intelligibility index (SII, Study I)

Each subject's real ear to coupler difference (RECD) was measured followed by measurements of HA gain at input levels of 55-, 65-, and 75-dB SPL. The input signal was the international speech test signal. Coupler verification with RECD was used instead of real ear verification with the HA on the ear, for time reasons. When the RECD was measured the subject could take a break from the testing.

An Aurical HIT test box was used (OTO suite, GN Otometrics, Denmark). The aided and unaided speech intelligibility index (SII) was calculated according to estimate speech audibility, by using the unaided PTTs and the HA acoustic coupler gain at 65 dB SPL. The

SII is a value between 0 (no intelligibility) and 1 (100% intelligibility) that is highly correlated with intelligibility of speech (ANSIS3.5, 1997). Datalogging was also recorded for each participant.

4.3.5 Sound localization accuracy (SLA, Study I)

Horizontal SLA was measured with an objective eye-tracking system, using a fast (≈3 min) method with high reliability (Asp et al., 2016). The subjects were seated facing 12 active loudspeaker/video display pairs placed equidistantly in the frontal horizontal plane (±55°, loudspeakers at ear level 1.2 m in front of the subject, Figure 7). The subjects watched a movie as presented via one of the loudspeaker/display pairs. The sound shifted from one loudspeaker to another during the test, with a 1.6 sec sound-only period before reintroduction of the visual stimulus at the new sound location. An eye-tracking system (Smart Eye Pro, Gothenburg, Sweden) was used to record subject's gaze. The coordinates of the video displays and loudspeakers were defined in three dimensions in the eyetracking system. The subject's perceived azimuth was defined as the pupil's position relative to the active loudspeaker. SLA was quantified by an error index (EI) (Asp et al., 2011; Gardner & Gardner, 1973):

$$EI = \frac{\sum_{p=1}^{P} |i_p - k_p|}{\left(\sum_{p=1}^{P} \sum_{j=1}^{n} |i_p - j| \right) / \mathrm{n}} \qquad (6)$$

where *P* is the number of presentations with at least 3 recorded gaze samples in the 500 msec sampling period (P ≤ 24 in the current test paradigm), i_p is the presented loudspeaker (1 to 12) and k_p is the perceived azimuth (1 to 12) at the *p*th presentation. The number of loudspeakers (12) is *n*. EI = 0 corresponds to a perfect match between perceived and presented azimuths. EI = 1 corresponds to pure guess (0.72-1.28 95% CI for the current test paradigm). For further details of the setup and procedure, see (Asp et al., 2016; Johansson et al., 2020a). Each subject participated in three subsequent sessions: aided, unaided (randomized order), and unaided retest.

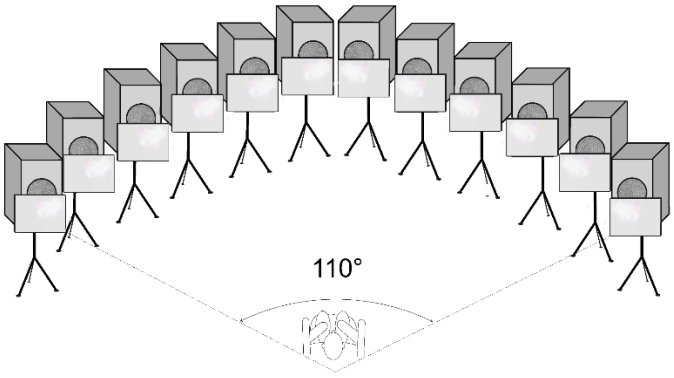

Figure 7. The sound localization setup. Illustration by Marlin Johansson.

4.3.6 Speech recognition threshold (SRT) in competing speech (Study I)

Aided and unaided SRTs were recorded in the presence of interfering speech, resembling a demanding everyday listening situation. The setup consisted of five loudspeakers where female target speech was presented from a loudspeaker in front of the subject at ear level. The subject was seated in the center of the room. The target speech was five-word sentences (Hagerman, 1982). Interfering speech was taken from a recording of a male speaker reading a novel. Four different sections of the recording were presented from each of four loudspeakers positioned at ±30° and ±150° azimuth at ear level (Asp et al., 2018; Berninger & Karlsson, 1999). Each subject participated in three subsequent sessions: aided, unaided (randomized order), and unaided retest. For full details on the adaptive method see (Asp et al., 2018; Hagerman, 1982; Hagerman & Kinnefors, 1995).

4.3.7 The Abbreviated Profile of Hearing Aid Benefit (APHAB, Study I)

A Swedish version of APHAB was used to quantify hearing disability (Cox & Alexander 1995) (Cox & Alexander, 1995). The APHAB consists of 24 items divided into four subscales: ease of communication, reverberation, background noise, and aversiveness of sounds. The subject responded to how frequently each situation occurred: always, almost always, generally, half-the-time, occasionally, seldom, or never. The questionnaire was administered twice, with an aided and unaided condition in mind.

4.3.8 The Parents' Evaluation of Aural/Oral Performance of Children (PEACH, Study I)

A Swedish version of PEACH was used to estimate the hearing performance of the children in everyday life (Brännström et al., 2014; Ching & Hill, 2007). The PEACH rating scale comprises 13 items and the result is presented in two subscales (quiet and noise) and as a total. The parent answered how frequently each situation (item) occurred on a five-point scale ranging from 0 to 4 (from never to always). The parent answered the PEACH rating scale with an aided and unaided condition in mind, starting in the aided condition.

4.3.9 TEOAE (Study II and III)

The TEOAEs in Study II (years 1998-2004) and Study III (years 2019-2020) were measured many years apart but used similar materials and methods. TEOAEs were recorded in the non-linear quickscreen neonate diagnostic mode with Echoport ILO288 (Otodynamics Ltd., UK). Program version 5.6 was used in Study II, and an updated program version 6 was used in Study III. A non-linear stimulus paradigm was used to record TEOAE stimulus and response levels at the probe tip in the outer ear canal with an electrically constant stimulus described elsewhere by Kemp et al. (1990). The stimulus levels corresponded to a median of 81.8 dB SPL peak (n = 60 431 ears) (Berninger, 2007).

In study III the interface was placed together with the patient in an audiometric test room for recordings with minimal background noise interference, while the computer and tested was placed outside in the surrounding test room. The left ear was tested first in 58% of neonates in study II and in 40% of infants in study III. The first tested ear may be important, as one study has found TEOAEs to be larger in the ear tested first (Thornton et al., 2003).

4.3.10 DPOAE (Study III)

The DPOAEs were recorded with the same test setup as for the TEOAEs with Echoport ILO288 USB-II (Otodynamics Ltd., Hatfield, UK, program version 6). The 2f1-f2 cubic distortion product component was measured with a frequency ratio of the primaries of 1.22 (f2/f1). The stimulus consisted of two equal-level sinusoids with an expected sound pressure level of 75 dB SPL at the tympanic membrane. The DPOAEs were recorded with f2 at 1, 1.5, 2, 3, 4, 6 and 8 kHz. The SNR was defined as the DPOAE level – (Noise + 2 SD).

4.3.11 MRI (Study III)

All but one of the 14 MRI scans were assessed with 3T scanners (Siemens Skyra or Siemens Prisma), the remaining with a 1.5T scanner (GE Optima). For all 3T scans, a protocol designed for pre-cochlear implantation was used, while a protocol designed for the temporal bone was used for the 1.5T scanner (for full protocols see (Johansson et al., 2022)).

Subjects were excluded from MRI if they were too ill for sedation with dexmedetomidine, which was determined in two steps, first by the responsible otologist, then by the MRI team physician.

The MRI results were reviewed by one or two experienced neuroradiologists or head-neck radiologists, and later all results were double checked by an experienced head-neck radiologist.

4.3.12 Congenital CMV infection testing (Study III)

A blood sample was taken from the mother for CMV testing as soon as possible after the study inclusion. If the mother had a positive CMV-test (IgG and/or IgM antibodies) the child's newborn dried blood spot (DBS) card was then analyzed for CMV DNA with the polymerase chain reaction (PCR) technique.

4.3.13 Genetic Testing (Study IV)

Fifteen subjects underwent comprehensive genetic testing using the OtoSCOPE◦ v.9 Platform. OtoSCOPE© v.9 uses targeted genomic enrichment and massively parallel sequencing of 224 HL-associated genes (for full list see supplementary material by (Johansson et al., 2023)). Four subjects were tested with four different gene panels at Karolinska University Laboratory, Karolinska University Hospital, due to malformations. Three subjects had malformations detected before or at birth (anal atresia, finger anomaly, corpus callosum agenesia, and tetralogy of Fallot, a heart defect). The fourth subject was identified with bilateral EVA with MRI at 7 months of age. The panels screened for chromosomal abnormalities or 105–137 genes (for full list see supplementary material by (Johansson et al., 2023)). Blood was typically collected from subjects after the MRI scan or after hearing test follow-ups at Huddinge, Sweden. All DNA samples were mailed at the same time to Molecular Otolaryngology and Renal Research Laboratories (MORL), USA for analysis. The mailing of all samples is why some of the subjects were tested with the Karolinska University Laboratory gene panels, as the responsible physician decided they needed a faster genetic result and could not wait for all samples to be collected. Genetic testing results were discussed at a multidisciplinary meeting with the MORL expert group consisting of geneticists, bioinformaticians, graduate students, auditory research scientists and otolaryngologists to determine the likely genetic cause of deafness, if any, for each subject.

4.3.14 Swedish Early Communication Development Inventory III (SECDI III, Study IV)

The first section of the Swedish Early Communication Development Inventory III (SECDI III) was used to obtain additional general information regard the study group of study III and IV. The first section of SECDI III was filled in by all 39 parents with child custody (the 20 infants' parents). The doctoral student did a free English translation of the information, as support for the parents that felt unsecure about answering in Swedish. The information included number of siblings, known language disorders, functional disability or other health issues, languages spoken in the home, infant's best language, and parental education as an indicator of socioeconomic status. All 39 parents first filled in the questionnaire when the child was 0.5-2.5 years old. The questionnaire was then filled in a second time at 2.5 years of age as a re-test (by 75% of the parents so far, with a child old enough). The first time the questions were administered the parent/parents that answered the questions was/were asked to fill in the education level of both parents. The

second time the questions were administered the parent that filled in the questions answered regarding only their own education level. The remaining questions were the same the first and second time.

4.4 Statistical analysis

All statistical analysis was performed with Statistica version 13 (Statsoft Inc., USA), or version 13.5 (TIBCO software Inc, USA), except linear mixed modelling in study III which was performed with R version 3.4.2 (R Foundation of Statistical Computing, Austria).

Generally, means and SDs were presented when a normal distribution could be assumed, and medians and interquartile ranges were presented when Kurtosis and Skewness differed from zero.

Various statistical methods were used in the thesis. Nonparametric tests were used when the distribution deviated from normal, and when small sample sizes were compared (Studies I, II, and III). When distribution did not deviate from normal parametric tests were used. Regression analysis and correlations were used to estimate relationships between variables in studies I, II, and III. Differences between correlation coefficients were computed using the Fisher's r-to-z transform, followed by an unpaired two-tailed t-test in study II.

Effect sizes for sex and ear differences were calculated as the difference between two means divided by the square root of the weight mean of the two variances (study II, Cohen's d). The effect sizes of 0.2, 0.5, and 0.8 were estimated as small, medium and large (Cohen, 1992). Effect sizes for the difference between correlations were calculated as the difference between two z-values, where effect sizes of 0.1, 0.3, and 0.5 are estimated as small, medium and large (Cohen, 1992). In study II a heritability model was also used (Falconer & Mackay, 1996; Fellman & Eriksson, 2006), as well as bootstrapping to estimate the variance in the heritability estimate.

In study IV a parametric proportions difference test was used, e.g., to compare infants with alleged syndromic uSNHL to infants with alleged non-syndromic uSNHL when it comes to the proportions with a genetic diagnosis. The *p*-value was calculated based on the *z*-value for the respective comparison:

$$|z| = \sqrt{[(N1 \times N2)/(N1 + N2)]} \times |p1 - p2|/\sqrt{(p \times q)} \qquad (7)$$

where $N1$ is the sample size of the first proportion ($p1$), and $N2$ is the sample size for the second proportion ($p2$), and:

$$p = (p1 \times N1 + p2 \times N2)/(N1 + N2) \qquad (8)$$

$$q = 1 - p. \quad (9)$$

4.5 Ethical considerations

Ethical approval was obtained for all studies (Study I: 2015/1878-31/2; Study II: 2019–03826; Studies III-IV: 2018/1500-31).

In studies I, II and IV written informed consent was obtained from all participating children's parents with child custody. In children who were determined to have the ability to give informed assent, this was obtained (Study I).

In study II TEOAEs were recorded in more than 30 000 newborns during the years 1998-2004 with optional participation. Before the measurement the parent(s) were informed orally that the results would be saved (e.g., for analysis if the subject later showed HL). The study II data was extracted from the TEOAE equipment for off-line analysis, where no personal ID numbers are saved (no last 4 digits). Thus, due to the large number of newborns included in the analysis (n = 454 twins and n = 21 199 non-twins) it would not be feasible to ask for written informed consent, as the risk of going through a vast number of medical records without personal ID numbers is both time consuming and difficult. Moreover, the results were only presented on group level, and only data that were relevant for the study design was extracted. These data comprised TEOAE levels, TEOAE reproducibility, SNR in different frequency bands, number of measured sweeps, sex of the child, and ear being measured. The only parameter that we deemed too sensitive to be kept together with the other data was the mother's name and the recording date. The data were needed to identify the twins, but coded in the files we used for analysis, and the original files kept separately, as the procedure for all studies.

The original data files and code-lists to connect the subject ID to the participant were kept in locked safe boxes in an alarmed corridor and/or a locked and alarmed room only available for authorized hospital personal, and keys available for the responsible researchers. The apparatus was placed in alarmed corridors and locked rooms when not used for clinical and research work, to avoid manipulation of information and systems. All pseudonymised data was stored on a Karolinska Institutet-approved server for secure storage of research data.

The sound levels of the apparatus needed to be considered for all studies and different age groups included in the studies, to obtain sensitive measurements without induced harm to the subject. All equipment has been appropriately calibrated before use. Objective methods were used as much as possible over subjective methods, to minimize bias.

Studies including children always have ethical concerns due to autonomy. This was a consideration when the 10-11-year-old children were asked in study I to give their informed agreement, as well as the parents. Especially newborns and infants included in research do not have the capacity of informed decision making. Thus, the parents take on the responsibility of making decisions for them.

The declaration of Helsinki (ethical principles for medical research involving human subjects) state that only if the research outcomes of a study have directly relevant research outcomes for a vulnerable group's health needs should they be included in research, and if a non-vulnerable group cannot be studied instead with similar research outcomes. The statement is in accordance with the included studies in the thesis. The recruited children with congenital uSNHL comprise the group that will benefit from the research outcomes. The congenital uSNHL causes are species specific, animal models cannot be used to study the causes for human uSNHL and related syndromes without first knowing the causes and mechanisms underlying the congenital uSNHL. To study HA outcomes in everyday life situations with animals and expect similar research outcomes as using the population is also unrealistic. Due to maturation and development, it is not possible to perform the studies of HA effects on adults and expect the same research outcomes. Moreover, it is not possible to determine if a congenital uSNHL was indeed congenital before the introduction of the introduction of NHS. We considered using an older group of children to study the causes and mechanisms of congenital uSNHL, as has been done for congenital bilateral SNHL (Berninger et al., 2022). However, the group that could be recruited with a similar study design was smaller than the group included in studies III-IV, and as the aim of finding the cause for uSNHL is already in accordance with what is done in the clinic for the infants, we determined that we could not obtain similar research outcomes with an older group of children. The study of causes and mechanisms is also part of a larger project in which the PhD student has collected longitudinal data as part of the PhD studies, where the research outcomes could not be studied with animal models or in adults or older children due to maturation and development. Moreover, knowing etiology is a great foundation for other relevant research outcomes for children with congenital uSNHL.

The thesis studies were overall designed according to the principles of the declaration of Helsinki. The rights and interests of the research subjects were considered in the studies in several ways. Timely information and time to consider participation in the studies were of importance, as well as giving both oral and written information, and additional time during and after oral information to answer questions and discuss concerns. The first weeks with a newborn are special and it may be a vulnerable period for parents. A late or absent diagnosis of, e.g., a syndrome may induce more long-term harm to a child compared to an early diagnosis. However, an early diagnosis may come during a time when the family is not prepared for the consequences, and this timing needs to be taken

into consideration in discussions with the parents. To avoid including subjects of ill health in the studies, clear inclusion and exclusion criteria were decided beforehand. Nonetheless, in infants it is difficult to discover all aspects of the child's health, and additional health issues may arise over time. We tried to make overviews of all possible MRI and genetic findings that may be diagnosed in congenital uSNHL beforehand, but the reason the research is needed is that there is not enough research. Thus, the parents were informed as well as possible before entering the study of what we may find, but also of the fact that we do the research as we do not know enough about congenital uSNHL. The studies of the infants were also designed to be connected to appointments and measurements that should be performed already in clinical work, to avoid taking extra and double time from the parents and infants, and the PhD student also made sure to be available for questions and contact throughout the studies.

5 Results

5.1 Hearing aid (HA) outcomes in children with congenital uSNHL

The six 9–10-year-old children participating in study I had used HAs for more than 1.5 years. They showed mean 4-frequency PTAs of 45 dB HL in their IEs (SD = 8 dB; 0.5-4 kHz). The shape of the audiograms varied within the group (Figure 8). The NEs showed a corresponding mean PTA of 6 dB HL (SD = 4 dB). The dataloggings showed an average HA usage time of 5.1 hours daily, although the variability was large from 0.7 hours to 12.7 hours.

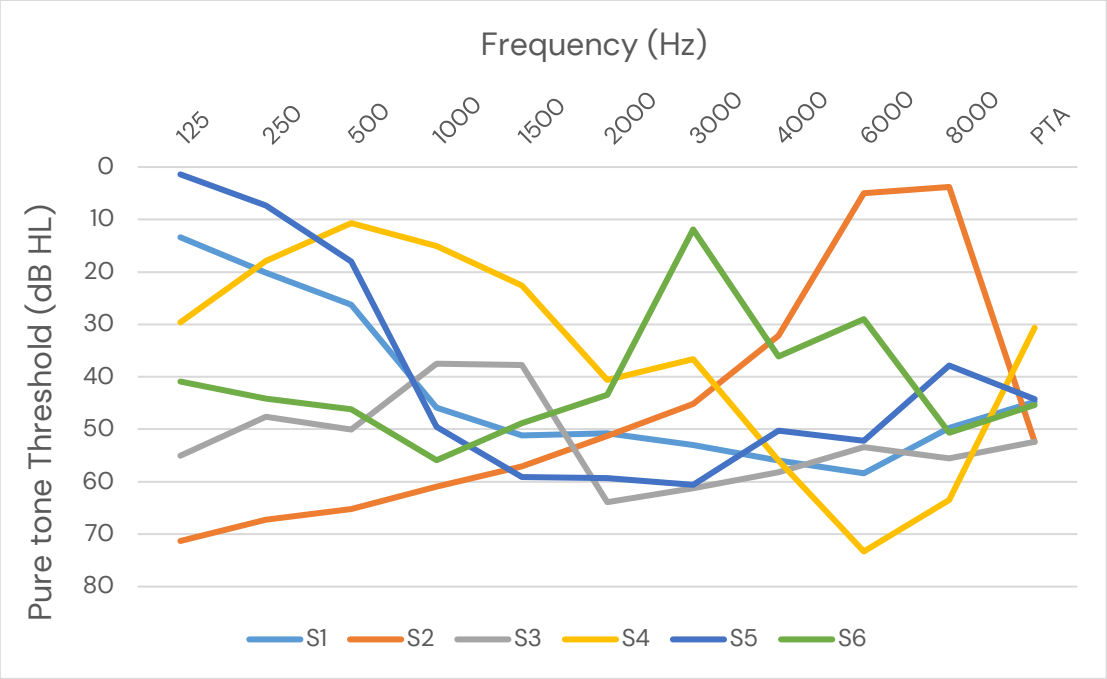

Figure 8. The pure-tone thresholds (PTT) versus frequency functions for subjects 1-6 (S1-6) in study I, as well as the pure-tone average (PTA) for each subject. Unpublished figure, with data from Johansson et al. (2020a).

5.1.1 School-aged children with uSNHL struggle in demanding listening situations (Study I)

The APHAB questionnaire (children) revealed higher perceived aided and unaided hearing disability compared to NH children (ps ≤ 0.001, n = 6 compared to n = 20, two-sided t-test) (Rance et al., 2014). The PEACH questionnaire (parents) showed a lower aided and unaided aural/oral performance compared to NH children (ps ≤ 0.01, n = 6 compared to n = 9, t-sided t-test) (Bagatto & Scollie, 2013).

The sound localization test demonstrated a lower aided and unaided SLA compared to NH adults (ps < 0.001, n = 6 compared to n = 8, two-sided t-test) (Asp et al., 2016). SLA is typically mature at 5-6 years of age, so we expected an adult-like response (Asp et al., 2016; Van Deun et al., 2009).

The mean SRTs in competing speech showed a significant impairment in the aided condition compared to young normal hearing adults ($p \leq 0.001$, n = 6 compared to n = 8, t-sided t-test) (Asp et al., 2018), with an 0.6 dB/year age correction (Berninger unpublished results, n = 48). However, the unaided SRTs did not reach a significant difference with the same comparison group.

5.1.2 HA benefit and dis-benefit (Study I)

Children with congenital uSNHL showed significant dis-benefit in horizontal SLA, based on a significantly worse aided than unaided SLA ($p < 0.05$, n = 6, Wilcoxon matched pairs test; Figure 9a). The three children with the most accurate unaided SLA (subjects 1, 4, and 5, Figure 9a) had the numerically lowest PTTs at 125 to 1000 Hz, although no statistically significant relationship could be established. No significant aided to unaided difference could be found in SRTs in competing speech ($p > 0.05$, n = 6, Wilcoxon matched pairs test), and the results showed large variability (Figure 9b).

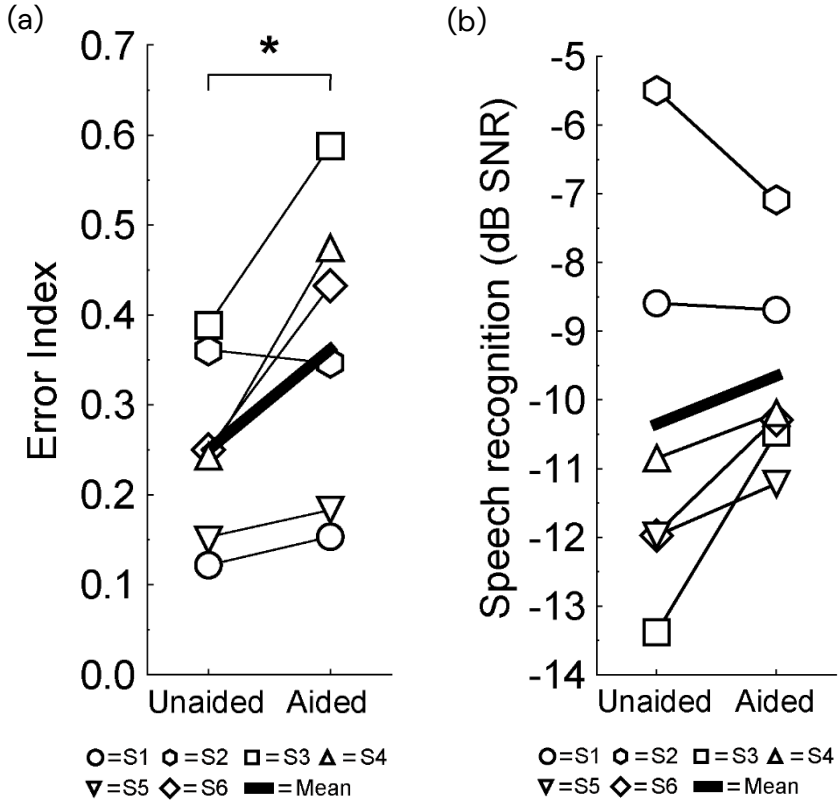

Figure 9 Hearing aid (HA) outcomes for the six subjects (S1-6). The thick solid line is depicting the mean; (a) An error index (EI) is quantifying the unaided and aided horizontal sound localization accuracy (SLA). EI = 0 means a perfect SLA, whereas an EI < 0.72 indicates a chance performance. (b) A signal-to-noise (SNR) is quantifying the speech recognition threshold (SRT) with a constant competing speech level of 63 dB SPL C_{eq}. Thus, a lower SNR indicates a better performance. Figures by (Johansson et al., 2020a) published open access by Wolters Kluwer Health, Inc.

In contrast to the psychoacoustic tests, the questionnaires showed HA benefit in overall PEACH aural/oral performance ($p < 0.05$, n = 6, Wilcoxons matched pairs test), and for the APHAB ease of communication (EC) subscale ($p = 0.03$, n = 6, Wilcoxons matched pairs

test). Neither HA benefit nor dis-benefit was found for the other APHAB subscales (Figure 10). The PEACH subscales (quiet and noise) showed numerically higher values in the aided condition, although failed to reach a significant difference between the conditions (noise subscale: p = 0.07, n = 6; quiet subscale: p = 0.12, n = 6, Wilcoxons matched pairs test) (Figure 11).

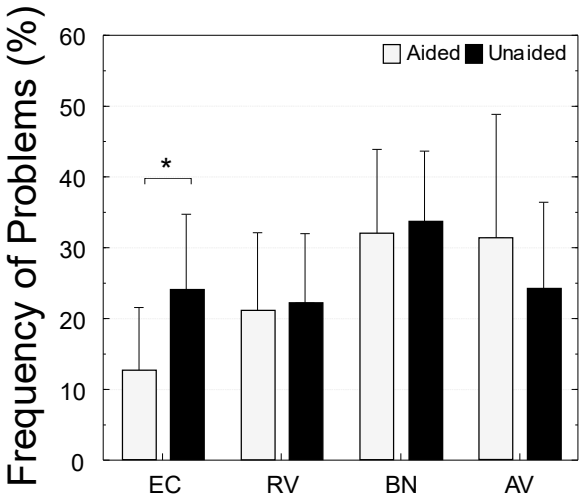

Figure 10. Hearing aid (HA) outcomes with the abbreviated profile of hearing aid benefit (APHAB) showing frequency of problems (i.e., hearing disability) (n = 6). Thus, a lower percentage is better for the child with uSNHL. Results are presented per subscale: EC = ease of communication, RV = reverberation, BN = background noise, AV = aversiveness of sound. Figure by (Johansson et al., 2020a) published open access by Wolters Kluwer Health, Inc.

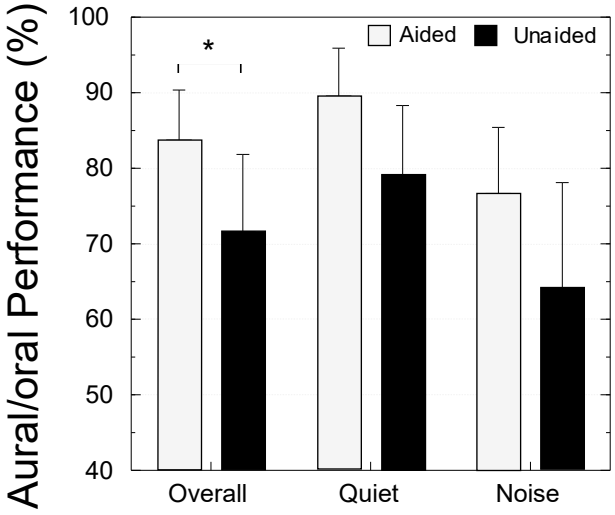

Figure 11. Hearing aid (HA) outcomes with parents' evaluation of aural/oral performance (PEACH) (n = 6). Thus, a higher percentage is better for the child with uSNHL. Results are presented as a total and per subscale. Figure by (Johansson et al., 2020a) published open access by Wolters Kluwer Health, Inc.

5.1.3 Neural transmission time and sound localization accuracy (SLA, Study I)

A linear regression analysis was performed to explore if the poor aided horizontal SLA result was reflected in neural activity up to the upper brainstem, where the auditory input

from the two ears cross-over. The ABR interpeak wave I-V interval is an indicator of the neural transmission time between the cochlea and upper brainstem (Eggermont & Don, 1986). A significant relationship between the aided EI and the ABR I-V interval was found (r = 0.98, p = 0.02, n = 4), (Figure 12). When the wave I from the NE was used to estimate an IE ABR I-V interval for a fifth subject that lacked an IE wave I, but showed an IE wave V, the close relationship remained (r = 0.98, p = 0.004). Linear regression between the absolute wave V latency and aided SLA also revealed a significant close relationship (r = 0.92, p = 0.03, n = 5), although the wave V latency less accurately reflect the neural transmission time, as it also includes non-linear cochlear processing affected by the degree of uSNHL. Nonetheless, with a correction factor for the degree of SNHL by (Jerger & Johnson, 1988) the aided SLA as a function of ABR wave V latency was still significant (r = 0.93, p = 0.02, n = 5).

No significant relationship was found between the unaided SLA and the ABR I-V interval in the IE (r = 0.63, p = 0.37).

No significant relationship was found between aided SLA and age at first HA fitting, although a trend towards a relationship could be observed (r = 0.79, p = 0.06, n = 6).

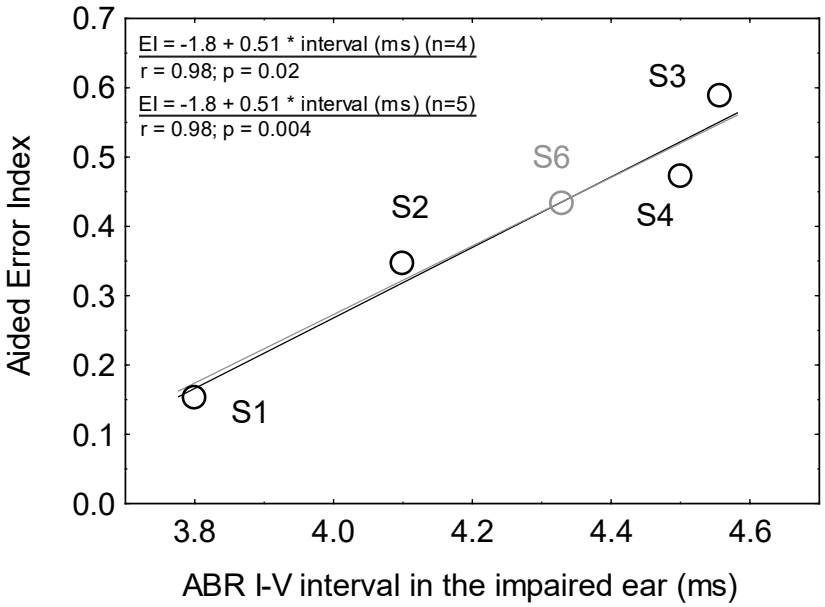

Figure 12. Linear regression analysis of aided horizontal sound localization accuracy (SLA) as a function of the IE ABR wave I-V interval. The SLA is quantified by an error index (EI) where a lower EI indicates better SLA. The black regression line and symbols depict the regression for the subjects with measurable ABR waves I and V. The grey regression line and additional symbol show the estimated regression including the fifth subject with an ABR wave V and an estimated IE wave I latency based on the NE ABR wave I latency. Figure by (Johansson et al., 2020a) published open access by Wolters Kluwer Health, Inc.

5.2 Heritability of neonatal transient-evoked otoacoustic emissions (TEAOEs, Study II)

Study II estimated TEOAE heritability for the first time based on neonatal TEOAEs by using twin subjects.

A significant effect was found on the TEOAE levels for sex ($F(1, 904) = 17.5$, $p < 0.0001$) and ear ($F(1, 904) = 17.1$, $p < 0.0001$) with factorial ANOVA. As expected, TEOAE levels were stronger in females (1.5 dB, n = 222, compared to n = 232 males), and right ears (1.5 dB, n = 227 right and left ears, twin pair average TEOAE level). The Cohen's d effect sizes for the ear and sex difference were 0.21–0.42, i.e., small (Cohen, 1992).

Both within-twin pair correlation coefficients of same-sex (SS) and opposite-sex (OS) twin pairs were significantly different from zero (Table 2). In contrast, when the twins were randomly paired, the correlation coefficient was close to zero for all ear comparisons (r = -0.04–0.06, n = 227 non-biological twin pairs, 454 twins) and when the subgroups were stratified for sex before randomization (r = -0.03–0.09, $p > 0.05$, n = 151 non-biological twin pairs of SS, and n = 76 non-biological twin pairs of OS).

A significant difference existed when comparing the correlation coefficient for the twin pairs of SS and OS for the ear-average TEOAE and L1–R2 + R1–L2 condition (Table 2). The L–L and R–R ear conditions did not reach significance, even if the correlation coefficients were numerically larger for the twins in SS pairs, compared to OS pairs. A difference was expected, as twins of OS are always DZ, whereas twins of SS can be either DZ or MZ.

Table 2. Within-twin pair correlations for TEOAE level. Comparison is made between correlations of twin pairs of the same-sex (SS, n = 151 pairs, 302 individual twins) and twin pairs of opposite-sex (OS, n = 76 pairs, 152 individual twins). For the L1–R2 + R1–L2 condition both ears of each twin were included in the analysis (SS, n = 302 ears; OS, n = 152 ears), whereas for the ear-average a mean TEOAE level was calculated first for each twin and the ear-average TEOAE level was correlated with the co-twin's ear-average TEAOE level (SS, n = 151 pairs; OS, n = 76 pairs). The differences between correlation coefficients were calculated using Fisher's r-to-z transform with a two-tailed t-test. Table by Johansson et al. (2020b), published open access by Elsevier, with slightly altered formatting than the original.

Twin 1 vs. Twin 2	**SS**		**OS**		r_{SS} **vs.** r_{OS}
	r	*p*	*r*	*p*	*p*
L–L	.48	< .0001	.30	.01	.14
R–R	.37	< .0001	.24	.03	.32
L1–R2 + R1–L2	.42	< .0001	.19	.02	.01
Ear-average TEOAE–Ear-average TEOAE	.52	< .0001	.27	.02	.04

L=left ear, R=right ear. Twin 1 was born before Twin 2.

Within-twin pair estimated MZ and DZ correlation coefficients were significantly different from zero (Table 3). As expected, a significant difference existed when comparing the

correlation coefficient for the estimated MZ twin pairs and DZ twin pairs for all ear conditions (Table 3), except for the right ears that failed to reach significance. The difference between estimated MZ and DZ correlations resulted in an effect size of 0.74, i.e., a large effect (0.5 = large) (Cohen, 1992).

Table 3. Estimated intra-twin pair correlation coefficients for monozygotic (MZ, estimated n = 75) and dizygotic (DZ, n = 76) twin pairs. For the L1-R2 + R1-L2 condition both ears of each twin were included in the analysis (MZ, estimated n = 150 ears; DZ, n = 152 ears), whereas for the ear-average, a mean TEOAE level was calculated first for each twin, and the ear-average TEOAE level was correlated with the co-twin's ear-average TEAOE level (MZ, n = estimated 75 pairs; DZ, n = 76 pairs. The differences between correlation coefficients were calculated using Fisher's r-to-z transform with a two-tailed t-test. Table by Johansson et al. (2020b), published open access by Elsevier, with slightly altered formatting than the original.

Twin 1 vs. Twin 2	**MZ**	**DZ**	r_{MZ} **vs.** r_{DZ}
	r	*r*	*p*
L-L	.62	.30	.01
R-R	.51	.24	.06
L1-R2 + R1-L2	.68	.19	.0001
Ear-average TEOAE- Ear-average TEOAE	.77	.27	< .0001

L=left, R=right. Twin 1 was born before Twin 2.

The estimated TEOAE heritability was $H^2 = 1.0$ (variance 0.33 calculated with bootstraping), indicating heritability of around 100%. The bootstraping analysis showed that 75% of the estimated H^2 exceeded 0.69.

Finally, previous twin research of OAEs (SOAEs, TEOAEs) has shown that a prenatal masculinization effect may exist for females in OS twin pairs (McFadden & Loehlin, 1995; McFadden et al., 1996). SOAEs were significantly fewer in females in DZ twin pairs compared to other females, with numbers more similar to the male's SOAEs (McFadden & Loehlin, 1995). The TEOAE levels were numerically weaker in females in DZ twin pairs compared to other females, although not statistically significantly weaker (McFadden et al., 1996). The effect was not present in the neonatal twin's TEOAEs, as the TEOAE levels were not weaker than the other female TEOAEs, instead numerically larger (Figure 13).

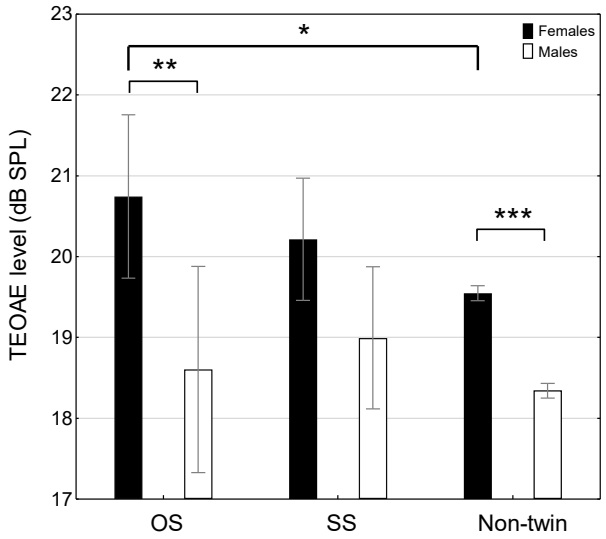

Figure 13. Differences in mean TEOAE levels (ear-average) grouped by sex and subgroup. Females in opposite-sex (OS) twin pairs (n = 76) did not show a prenatal masculinization effect, as the TEOAE levels were 2.1 dB larger than their male co-twins' TEOAE levels (n = 76). The figure also shows the mean TEOAE levels of same-sex (SS) twin pairs (n = 156 males, n = 146 females), and of non-twins (n = 10673 males, n = 10526 females). Vertical bars show 95% CIs, $*p < 0.05$, $**p < 0.01$, $***p < 0.0001$. A paired t-test was used to estimate the sex difference within OS-pairs. A Mann–Whitney U test was used to estimate the OS-twin vs. non-twin relationship. An unpaired t-test was used to calculate the sex difference in non-twins. Figure by Johansson et al. (2020b), published open access by Elsevier.

5.3 Etiology and auditory profiles of infants with congenital uSNHL

The twenty infants participating in study III and IV were diagnosed with congenital uSNHL at a median of 2.2 months of age. The degree of uSNHL varied from mild to profound with a median of 55 dB nHL (see all subjects' ABRthrs in Table 4). For subjects with recordable IE ABR waves the median ABR thr was 43 dB nHL (n = 12). All NEs showed an ABRthr of ≤20 dB nHL (n = 20).

One subject was born pre-term, the rest full-term. Six of the 20 infants stayed in the neonatal intensive care unit for several days (due to asphyxia, anal atresia, brain anomaly, jaundice, mild respiratory distress syndrome, or heart anomaly; Table 4). One out of the 20 infants had a first-degree family history of HL (a brother with uSNHL).

The initial questions of the SECDI-III questionnaire, used to obtain relevant clinical information about the infants and families, showed that most infants were exposed to more than one language in the home (55%; n = 11/20). Forty-one percent of parents had a ≥3 year university degree, similar to the national figure of 45% reported for 25-64-year olds across Sweden (Statistics Sweden, 2022).

5.3.1 A high prevalence of malformations in congenital uSNHL (Study III)

Nineteen subjects were eligible for MRI. One was excluded based on a heart anomaly.

Fourteen subjects underwent MRI, and 64% were diagnosed with a malformation (9/14) (Table 4). The malformation prevalence for infants with an IE ABRthr of 35-60 dB nHL (mild to severe uSNHL) was 43% (3/7 MRI scans). For the remaining with no recordable IE ABRthr (>90 dB nHL, indicating SSD), the prevalence was especially high at 86% (6/7 MRI scans). In mild to severe uSNHL inner ear malformations were most common, whereas for SSD cochlear nerve aplasia or hypoplasia was most common (Table 4).

In 50% of the infants with uSNHL no visible IE cochlear nerve was observed, i.e., cochlear nerve aplasia or hypoplasia was revealed (7/14 MRI scans; Figure 14). In 29% of infants an inner ear malformation was observed with imaging (4/14 MRI scans; Figure 14). Two subjects revealed a combined inner ear malformation and cochlear nerve aplasia or hypoplasia. The total percentage of EVA was 21% (3/14 MRI scans). Bilateral EVA was observed in one infant, and two infants revealed unilateral EVA (Figure 14). The subject with bilateral EVA had an ABRthr of ≤20 dB nHL in the NE, as well as TEOAEs and DPOAEs in the NE at 2 months of age. At a follow-up visit when the infant was 8 months old (MRI at 7 months) the uSNHL had deteriorated to a bilateral SNHL.

Table 4. Auditory brainstem response thresholds (ABRthrs), magnetic resonance imaging (MRI) results, and genetic findings. All subjects had ≤20 dB nHL ABR Thresholds (ABRthrs) in their normal-hearing ear (NE) at diagnosis (median age 2.2 months). Variant interpretation reflects Molecular Otolaryngology and Renal Research Laboratories (MORL) expert opinion and considers all extracted data from the Deafness Variation Database (DVD, http://deafnessvariationdatabase.org). Adapted version of Table 1 by Johansson et al. (2023), published open access by MDPI.

ID	IE	Sex	ABRthrs IE (dB nHL)	MRI result	Variants found in genes, with probable genetic cause for hearing loss	Possibly relevant clinical features or family history of hearing loss
1	L	M	45	--	--	Asphyxia with brain injury
2	L	F	35	0	GJB2, two variants at the DFNB1 locus, autosomal recessive non-syndromic hearing loss	
3	L	F	>90	Hypoplasia cochlea, aplasia/severe hypoplasia cochlear nerve, semicircular canal dysplasia and EVA	SALL1, one variant found for Towns-Brocks syndrome*	Anal atresia, finger malformation
4	R	F	40	--	--	Strabismus, slight stutter
5	L	M	40	0	0	
6	L	M	>90	Aplasia cochlea, aplasia/severe hypoplasia cochlear nerve, labyrinth dysplasia and semicircular canal dysplasia	0	
7	R	F	45	Bilateral EVA with probable IP II	SLC26A4, two variants, also found in parents, Pendred syndrome*	

8	R	F	>90	Aplasia/severe hypoplasia cochlear nerve and hypoplasia inner ear canal	0	
9	L	M	>90	--	0	
10	L	M	40	--	Chromosome 8P inverted duplication (8p11.1p23.1, ~6,9 Mb) and deletion (8p11.1p23.1, ~30,8 Mb) syndrome*	Corpus callosum agenesia
11	R	M	>90	Aplasia/severe hypoplasia cochlear nerve and hypoplasia inner ear canal	0	
12	R	F	>90	Aplasia/severe hypoplasia cochlear nerve and hypoplasia inner ear canal	0	Born small for age in week 36+1, NICU 1 week for jaundice
13	L	F	>90	0	0	Older brother with single-sided deafness >80 dB nHL, IE also L
14	L	M	>90	Aplasia/severe hypoplasia cochlear nerve	0	Born with mild respiratory distress syndrome, 3 days NICU, no apparent permanent effects
15	L	M	40	0	0	Twin
16	L	F	45	0	0	
17	L	M	60	Aplasia/severe hypoplasia cochlear nerve	0	Twin
18	R	F	60	Unilateral EVA with probable IP II	0	
19	L	M	40	--	CHD7, autosomal dominant CHARGE syndrome	Tetralogy of Fallot (congenital heart defect), feeding difficulties
20	R	F	50	--	0	

* Tested outside of OtoSCOPE® v.9 panel at the Karolinska University Laboratory. -- = no test; 0 = no anomaly detected; ABR = auditory brainstem response; ABRthr: auditory brainstem response threshold; EVA = enlarged vestibular aqueduct; IE = impaired ear; IP II = cochlear incomplete partition type II; L = left; MRI = magnetic resonance imaging; NICU = neonatal intensive care unit; R = right; uSNHL = unilateral sensorineural hearing loss.

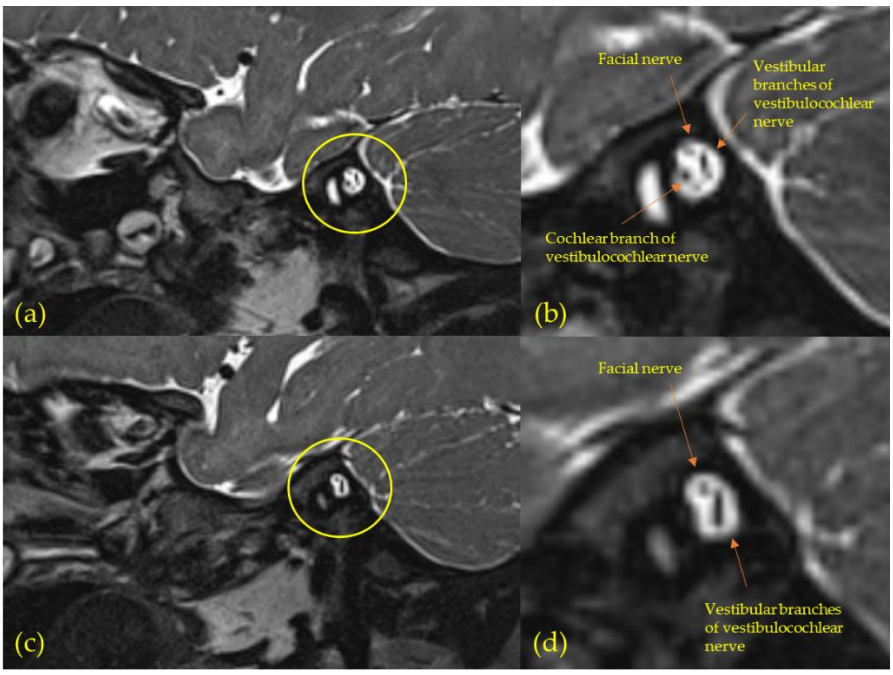

Figure 14. Magnetic resonance imaging (MRI) of the most common malformation in congenital unilateral sensorineural hearing loss (uSNHL), cochlear nerve aplasia or hypoplasia (oblique-sagittal view); (a,b) The normal-hearing ear (NE) of subject 11 (S11) is showing a typical cochlear branch of the vestibucochlear nerve; (c,d) The impaired ear (IE) of S11 is showing no visible cochlear nerve branch, indicating aplasia or severe hypoplasia. Figure by Johansson et al. (2022) published open access by MDPI.

5.3.2 None of the infants were congenital CMV infection positive (Study III)

All infants were congenital CMV infection negative (n = 20). This was based on analysis of 16 infant's DBS cards, one infant's plasma test the same day as birth, and three CMV IgG and IgM negative mothers (blood test 51-88 days after birth).

5.3.3 Genetic testing (Study IV)

A genetic cause for the uSNHL was found in 28% of the infants (n = 5/18 tested, Table 4).

All three infants with non-hearing related malformations observed at birth (of a hand, anus, brain, and/or heart) were diagnosed with a genetic cause. These causes included CHARGE syndrome (CHD7, tetralogy of fallot), Townes–Brocks syndrome (SALL1, anal atresia and finger malformation), and Chromosome 8P inverted duplication and deletion syndrome (corpus callosum agenesis) (Table 4).

Of the remaining subjects 2/15 were also diagnosed with a genetic cause for the uSNHL. One of these subjects demonstrated Pendred syndrome (SLC264A) with bilateral EVAs revealed with MRI (Table 4). The final subject demonstrated a non-syndromic uSNHL, i.e., autosomal recessive non-syndromic HL (GJB2 that causes a Connexin 26 mutation) (Table 4).

The genetic variants diagnosed as causes for uSNHL were only a small portion of all 198 genetic variants found with the genetic testing. Most genetic variants found with the OtoSCOPE© v.9 panel was variants of uncertain significance (VUS), 118 variants in all subjects tested, compared to only 5 pathogenic (P) variants, and 3 likely pathogenic (LP) variants (see percentages in Figure 15).

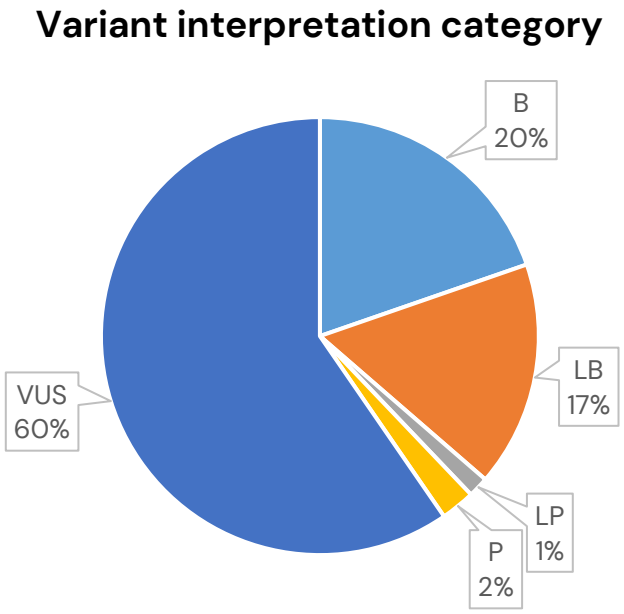

Figure 15. Variant interpretation category percentages from the results of the OtoSCOPE© v.9 panel with a total of 198 variants found (representing the total of 100%). Fifteen subjects were tested with the OtoSCOPE© v.9 panel (University of Iowa), and four subjects with other genetic panels (Karolinska University Laboratories). In total, 18 subjects were genetically tested. Interpretation reflects Molecular Otolaryngology and Renal Research Laboratories (MORL) expert opinion and considers all extracted data from the Deafness Variation Database (DVD, http://deafnessvariationdatabase.org) (Azaiez et al., 2018). Unpublished figure with some of the data presented by Johansson et al. (2023), published by MDPI. B = benign; LB = likely benign; LP = likely pathogenic; P = pathogenic; VUS = variant of uncertain significance.

The total diagnostic yield of MRI or genetic testing was 67%, i.e., a cause for the congenital uSNHL was found in 12 out of 18 infants tested. In the 14 infants tested with both MRI and genetic testing the diagnostic yield was 71%, with a diagnosis in 10 infants. Two of the infants had a congenital uSNHL that revealed both a malformation with MRI, and a genetic cause for that malformation (Table 4).

Four out of five infants with a genetic cause for the congenital uSNHL demonstrated a mild to moderate uSNHL with ABR at diagnosis (ABRthr of 35-45 dB nHL). The remaining subject with a genetic diagnosis showed SSD with a ABRthr >90 dB nHL.

5.3.4 Auditory profiles and affected hearing mechanisms (Study III)

The results from bilateral TEOAEs, DPOAEs and tympanograms corroborated the diagnosis of congenital uSNHL.

Further supporting the diagnosis of congenital uSNHL, the ABR latency as a function of stimulus level was similar in the two ears (for wave I, III, and V; ps > 0.05, Mann-Whitney U test).

No significant interaural difference was found in ABR wave I-V intervals (p = 0.26, n = 9, Wilcoxon's matched pairs test), indicating no interaural neural conduction difference between the cochlea and upper brainstem.

Linear mixed modelling showed a significant effect of ear (NE vs. IE) on the ABR amplitudes of wave I ($r_{total\ model}$ = 0.64, p < 0.001), wave III ($r_{total\ model}$ = 0.76, p < 0.001), and wave V ($r_{total\ model}$ = 0.78, p < 0.001). Not surprisingly, stimulus level also showed a significant effect on the ABR amplitudes (p < 0.001 wave I, p < 0.001 wave III, and p = 0.004 wave V). The effects are demonstrated in the parallel shifted NE vs. IE input/output (I/O) function for wave I, III and V (Figure 16). The parallel shift, together with a significant interaural difference in ARTs indicates an absence of loudness recruitment by neural firing on group level (Eggermont, 1977; Karlsson et al., 1995).

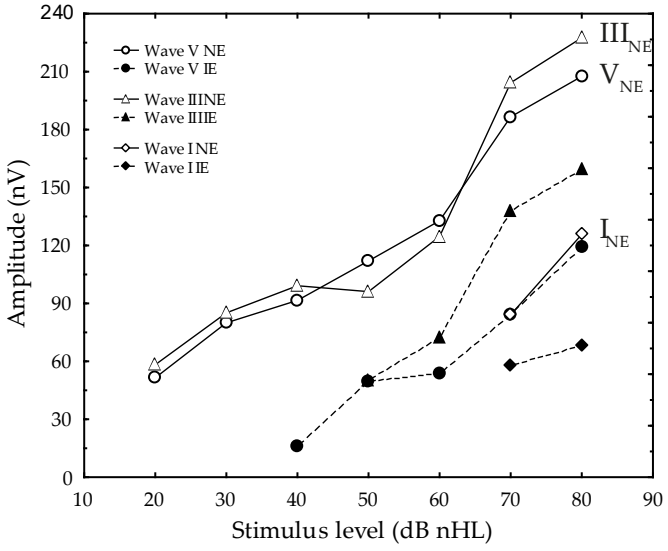

Figure 16. Mean auditory brainstem response amplitude as a function of stimulus level (dB nHL) for wave I (rhombi), wave III (triangles), and wave V (circles). The IE (filled) vs NE (open) functions show a parallel shift for all waves. The IE wave I ns = 8 and 7 and NE wave I ns = 17 and 8 for 70-80 dB nHL, respectively. The NE wave III ns = 11, 12, 14, 11, 9, 20, and 9 for 20-80 dB nHL, respectively, whereas the IE ns = 7, 5, 11, and 10 for 50-80 dB nHL, respectively. The wave V NE ns = 20, 15, 16, 13, 9, 10, and 9 for 20-80 dB nHL, respectively, whereas the IE ns = 6, 10, 9, 12, and 11 for 40-80 dB nHL, respectively. Figure by Johansson et al. (2022) published by MDPI.

6 Discussion

6.1 Children with congenital uSNHL and HA implications

6.1.1 Children with congenital uSNHL compared to children with NH (Study I)

The results from study I demonstrate that 10–11-year-old children with uSNHL struggle in demanding listening situations with and without amplification. Notwithstanding a (statistically) relatively small group of six children with congenital uSNHL, aided and unaided subjective hearing disability (APHAB, children) and subjective aural/oral performance (APHAB, parents) were significantly impaired compared to age-matched children with NH (Bagatto & Scollie, 2013; Brännström et al., 2014; Rance et al., 2014) (Figures 10-11). Moreover, aided, and unaided SLA, as well as aided SRTs in competing speech were statistically significantly impaired compared to age-matched NH materials (Asp et al., 2018, n = 8, Asp et al., 2016, n = 8; Berninger unpublished results, n = 48) (Figure 9). It has been known for decades that children with uHL who do not use HAs generally have impaired speech understanding in noise and competing speech (Bess et al., 1986; Bovo et al., 1988; Johansson et al., 2020a; Ruscetta et al., 2005) and impaired SLA (Bess & Tharpe, 1984; Bess et al., 1986; Humes et al., 1980; Newton, 1983), even if the variability is large. Yet, it has not yet been shown (until now) that children who have been using HAs for more than 1.5 years display statistically significant impairments based on questionnaires and measurements of SLA and SRTs in competing speech. The previous studies of HA outcomes in uHL have focused on aided vs. unaided performance (Benchetrit et al., 2022; Briggs et al., 2011; Johnstone et al., 2010; Updike, 1994). Due to the lack of outcomes studies in uSNHL after early HA fittings, it is still unknown if the significantly poorer results compared to children with NH were due to HAs fitted late in development (Johansson et al., 2020a).

6.1.2 HA dis-benefit in sound localization accuracy (SLA, Study I)

HA disbenefit in SLA has been found in a previous study of children with uSNHL who were about the same age (10-14 year of age, n = 6 (Johnstone et al., 2010), vs. 10-11 years of age, n = 6 (Johansson et al., 2020a)). In both studies the children had been using HAs for some time (at least 3 months (Johnstone et al., 2010), and at least 1.5 years (Johansson et al., 2020a). All children 10-14 years of age were also fitted late in development, after the age of 5 years (Johansson et al., 2020a; Johnstone et al., 2010).

In contrast, the 6-9 year old comparison group with uSNHL included by Johnstone et al. (2010) (n = 6), were fitted with HAs earlier (4-6 years of age) and demonstrated HA benefit in SLA. Thus, the difference in HA benefit between the groups may be explained by HA fitting age, as a correlation was found between age at HA fitting and unaided-aided SLA ($r = -0.67$, $p < 0.05$, $n = 12$) (Johnstone et al., 2010). More specifically, the benefit may be explained by younger children's more plastic brains that may better adapt to altered

binaural cues introduced by a HA. Bilateral localization cues do not seem to be preserved after HA intervention, at least based on the results of children and adults with bilateral SNHL and bilateral HAs (Gorodensky et al., 2019; Van den Bogaert et al., 2006), and especially for interaural time difference cues (Gorodensky et al., 2019). Due to the delay of 5-10 ms introduced by a HA, as discussed by Johansson et al. (2020a), the detrimental effects are expected to be even larger for uSNHL. In study I we found no correlation that significantly deviated from zero for unaided-aided SLA in relation to the age at HA fitting, which presumably is explained by less variability in both age and age at HA fitting. In the 10-14 year old group by Johnstone et al. (2010), all showed a negative unaided-aided SLA (n = 6), i.e., a numerically worse aided SLA compared to unaided in all older children, and no apparent correlation between age at HA fitting and unaided-aided SLA within in this older age group (Figure 3 by Johnstone et al. (2010)). In study I the relationship between aided SLA and age at first HA fitting was not significantly different from zero, although the relationship was not far from reaching statistical significance ($r = 0.79$, $p = 0.06$, $n = 6$).

Another result corroborated the theory of increased aided SLA benefit with earlier HA fittings due to more plastic brains adapting better to HAs. A distinct relationship was found between aided SLA and the ABR wave I-V interval ($r = 0.98$, $p = 0.02$, $n = 4$, and 0.98, $p = 0.004$, $n = 5$; Figure 12). A close relationship was also found between aided SLA and the wave V latency ($r = 0.92$, $p = 0.03$, $n = 5$; $r = 0.93$, $p = 0.02$, $n = 5$ with correction factor (Jerger & Johnson, 1988)). Both the ABR and the SLA were measured with objective measurements, and the ABR determination of waves was blinded, to minimize bias. A longer ABR wave I-V interval may reflect a less developed neural function, which may have led to inefficient integration of binaural cues in the aided SLA condition. The upper brainstem, where the wave V is generated in humans (Møller & Jannetta, 1983; Parkkonen et al., 2009), has shown to be important for integration of spatial cues in cats (Chase & Young, 2005). Furthermore, detrimental effects on neural tuning for binaural integration has been demonstrated in rats and cats following monaural deprivation due to induced mild-to-moderate uHL (Clopton & Silverman, 1977; Moore & Irvine, 1981; Popescu & Polley, 2010; Silverman & Clopton, 1977). Consequently, it is not surprising that a child with congenital uSNHL that has some extent of monaural deprivation may have difficulty adapting to altered binaural cues.

The PTTs at low frequencies probably have an important role in the HA fitting of children with congenital uSNHL as well, although further study in larger samples is needed to establish the statistical relationship. Our results showed that the three subjects with the lowest (i.e., best) PTTs at low frequencies showed the most accurate unaided SLA, although not the three most accurate aided SLA results, indicating that the parameters in the fitting of the HAs are important. Open ventilation may be a favorable option for the children with congenital uSNHL and efficient low-frequency hearing, to gain access to

interaural time difference cues, that are important in horizontal SLA in NH (Wightman & Kistler, 1992).

6.1.3 HA benefit in one-to-one communication (Study I)

HA benefit was found in one-to-one communication, according to APHAB (EC subscale) filled in by the children with congenital uSNHL. Neither benefit nor dis-benefit was found in noisy and reverberant listening situations (BN, RV, and AV subscales, Figure 10). PEACH showed overall HA benefit, but not for the separate noise and quiet subscales (Figure 11). PEACH is designed for younger children than APHAB and focused on interactions with the parent, i.e., the questionnaire is more focused on one-to-one communication than APHAB, which may be one reason why the overall performance showed significant benefit. APHAB did not show benefit in more demanding listening environments (i.e., BN, RV subscales). The most positive HA outcomes for children with uHL generally come from questionnaire results (Benchetrit et al., 2022; Briggs et al., 2011; Johansson et al., 2020a). Two previous HA trials reported HA-benefit in home and school by the parent, child and teacher, and quality of life based on questionnaire results (Benchetrit et al., 2022; Briggs et al., 2011) (CHILD, LIFE, and HEAR-QL questionnaires). Benchetrit et al. (2021) only reported the overall five-questionnaire benefit, by using mean averaging of all survey's standardized scores, and Briggs et al. (2011) showed overall questionnaire scores with no division by noise and quiet or one-to-one and more demanding listening situations. However, the LIFE questionnaire included mostly noisy and difficult situations, and the results by the children indicated less listening difficulties in school situations with the HAs, compared to before the HA trial (Briggs et al., 2011).

The two HA trials included children with conductive, mixed and sensorineural uHL (Benchetrit et al., 2022; Briggs et al., 2011), in which most subjects included by Benchetrit et al. (2022) had conductive uHL (59%), which may partly explain why no detriments in noise were reported. Conductive uHL is caused by an issue with sound conduction, while SNHL affect cochlear and neural processing (Gelfand, 2016; Moore, 1996). HA trials using subjective measurements also have risks of placebo effect, depending on how the new intervention is presented to the person with HL (Dawes et al., 2013; Dawes et al., 2011). How the potential HA benefits (and/or dis-benefits) were presented to the subjects in was described in neither study (Benchetrit et al., 2022; Briggs et al., 2011), nor was there any equivalent intervention for comparison, as in a randomized control trial. Benchetrit et al. (2021) included cross-over with a frequency-modulated system and strategic seating in one arm, and the addition of a HA in arm 2 (about half the subjects started in arm 1, and the others in arm 2). However, as these alternatives cannot be estimated as equal, the risks of placebo effects and bias remain with the new intervention.

In study I the APHAB questionnaire was administered to the children with congenital uSNHL after the SRT in competing speech was measured, but before they knew the result

of the test. Many of the children expressed that they found the test difficult, which may have contributed to thoughts around which situations are more difficult with and without the HA in everyday life, in comparison to the studies that only used subjective measurements.

6.1.4 Neither HA benefit nor dis-benefit in competing speech (Study I)

The SRTs in competing speech showed neither significant HA benefit nor dis-benefit (Figure 9B), corroborating the previous studies of SRTs in noise in children with uHL and HAs (Briggs et al., 2011; Updike, 1994), and the questionnaire results (Johansson et al., 2020a).

6.1.5 Affected mechanisms in uSNHL with implications for HA fittings (Study III)

In infants with congenital uSNHL parallel shifted ABR I/O functions were found for the NE vs. the IE (Figure 16), supported by a significant effect of ear and stimulus level on the ABR amplitudes of waves I, III, and V in the linear mixed model. If the infants would have had cochlear HL with recruitment, believed to include most SNHL (Gelfand, 2016; Popelka et al., 2016), the amplitudes would have been similar between ears at higher input levels (Eggermont, 1977; Karlsson et al., 1995).

The association between the ABR I/O function and recruitment has been found in adults with recruitment previously (Eggermont, 1977), and with temporary OHC-associated cochlear HL in combination with unchanged (i.e., similar) ARTs in the two ears (Berninger et al., 1998; Karlsson et al., 1995). Thus, the lack of recruitment was supported by the significant difference in ARTs. If recruitment is present, ARTs at similar thresholds in the two ears are expected (Karlsson et al., 1995; Roeser et al., 2007). The apparent lack of recruitment at group level may have important implications for the HA fitting of children with uSNHL, that needs further research in older research groups with congenital uSNHL, and/or with non-electrophysiologic measurements of recruitment. Because of the many malformations found in congenital uSNHL, it may be that different HA amplification settings should be considered for congenital uSNHL compared to bilateral uSNHL, where the malformation incidence is much lower (Berninger et al., 2022; Johansson et al., 2022; Masuda & Usui, 2019; McClay et al., 2008).

6.2 Heritability of TEAOEs (Study II)

Neonatal TEOAE levels are largely inherited, corroborating a previous study in young adult twins revealing a similar result (McFadden et al., 1996). Our results showed a heritability estimate of 100% (where 75% of all estimates of heritability was above 69% with bootstrapping), numerically higher than the heritability estimate of about 75% in young adult twins (McFadden et al., 1996).

The large influence of heritability was evident when comparing the correlation coefficient between twins in OS pairs with twins in SS pairs (Table 2), and even more evident when comparing the correlation coefficients of twins of DZ zygosity to the correlation coefficients estimated for MZ twin pairs (Table 3). Further supporting that the close significant correlations for twins were not due to coincidence, the correlation coefficients for randomly paired twins were close to zero, even when a stratification for sex was included due to sex differences in TEOAE. The large heritability of TEOAEs also showed that TEOAEs are nonrandom events. The similarities between twin's TEOAEs were in stark contrast to the large between-individual variability in neonatal twins in general (Berninger, 2007; Bray & Kemp, 1987).

The heritability estimates by both study III and McFadden et al. (1996) were based on intra-twin pair correlations. A difference in the estimates was the use of ear average TEOAE correlations (Johansson et al., 2020b), and same-ear TEOAE correlations (McFadden et al., 1996). In the comparison between the TEOAE in different ears, we found that the estimated heritability was larger for opposite ears, than for same ears, due to a large difference between the estimated correlation coefficient of MZ twins compared to DZ twins (Table 3). The opposite ears of young adult male MZ twin pairs also revealed the largest correlation coefficient of r = 0.83 in the study by McFadden et al. (1996). Mirror-image twinning may be a theory explaining the high correlation coefficients for the opposite ears of MZ twins, i.e., that MZ twins often show mirrored features. Mirror-image twinning is a result of late zygotic splitting (Hall, 2003; McNamara et al., 2016), and exists in up to 25% of MZ twin pairs, as demonstrated by mirrored eye and ear defects (McNamara et al., 2016; Springer & Searleman, 1978). The effect of mirror-image twinning that may overestimate heritability was minimized with the use of the correlation coefficients based on the TEOAE ear average.

The largest difference between the study methods in young adult twins (McFadden et al., 1996) and neonatal twins (Johansson et al., 2020b) was the estimations of zygosity. Heritability in neonates was estimated based on twins in OS twin pairs always being DZ, with a mathematical model to estimate the correlation coefficient for MZ twins based on the data from the twins of SS and OS. A questionnaire procedure was used for young adult twins (Nichols & Bilbro, 1966), with about 90% accuracy in determining zygosity (McFadden & Loehlin, 1995). Thus, it would be of value if future research included DNA analysis methods for zygosity, that may estimate zygosity even more precisely.

Both studies of neonatal and young adult twins used a mathematical model for the heritability estimate. Heritability models are generally associated with large standard errors, but the higher the estimate (closer to 100%), and the larger the sample size, the more exact the estimate becomes (Falconer & Mackay, 1996). Using a simpler model, that MZ twins share 100% of their genes, it may be argued that the estimated MZ correlation coefficients of r = 0.51-0.77 for various ear combinations (Table 3) would indicate a

heritability of about 70-75% as for young adult twins. However, the DZ twins shared 50% of their genes, so MZ twins were on average twice as similar as DZ twins, which is the basis for Falconer's formula used. The formula assumes equal contribution of environmental factors in MZ pairs and DZ pairs, however, only basing the estimates of MZ twins does not take environmental factors into account at all. That TEOAEs are entirely inherited can be argued, but our results strongly suggest that they are largely inherited. We used bootstrapping to estimate the variance in our heritability estimate by resampling different subsamples of the twin data 10 000 times. Of the 10 000 heritability estimates we found that 75% of the heritability estimates exceeded 69%. Moreover, Cohen's effect size of 0.74 was notably large, as 0.5 indicates a large effect (Cohen, 1992).

Other method differences between the studies were the recording window. A non-linear mode with 3 ms onset was used in study II, whereas linear averaging after 6 ms was used in young adult twins (frequency components over about 3 kHz will probably be omitted (Keefe, 2012; Kemp, 1986)). The TEOAE levels were also 8 dB larger in neonatal twins compared to young adult twins. The TEOAE level difference mainly reflects two factors. The first being the difference in stimulus levels between the studies (about 20 dB stronger and nonlinear in neonates, where the lower stimulus level may enhance the detection of small TEOAE differences). The second being the age of the participants, mainly due to differences in ear acoustics TEOAEs are up to 10 dB larger in neonates (Ferguson et al., 2000; Kemp et al., 1990).

6.2.1 TEOAE sex and ear differences (Study II)

The significantly stronger TEOAEs in right ears compared to left ears (1.5 dB) and females compared to males (1.5 dB) have previously been found in adult twins (McFadden et al., 1996) and large groups of neonates (Aidan et al., 1997; Berninger, 2007; Kei et al., 1997; Thornton et al., 2003). TEOAEs typically recorded 3 days after birth, show that ear and sex differences are present from birth, as reported by Berninger (2007). The twin's TEOAEs were recorded at the first TEOAE occasion in 99% of cases, indicating that they were indeed recorded days after birth, and not at a later TEOAE visit. The 1.5 dB difference may not be clinically important, partly due to the large variability in TEOAEs, which was also reflected in a small effect size of 0.21-0.42 for the twins and co-twins by Cohen's d. Significant effects of both ear and sex have not been recorded in samples of less than 500 neonates before (twin group). Only an effect of ear was found in 483 preterm neonates (Khalfa et al., 1997), only an effect of sex in 350 neonates (Cassidy & Ditty, 2001), and no effect in 100 neonates (Johnsen et al., 1988). The effects we observe in the 454 twins are probably largely explained by good recording conditions with a median of 9.6 dB in TEOAE SNR, and the strict inclusion criteria in the study.

6.2.2 No masculinized TEOAEs in female twins of OS (Study II)

The female twins of OS did not show masculinized TEOAEs, contrary to the twin testosterone transfer hypothesis (McFadden, 1993a; McFadden & Loehlin, 1995; McFadden et al., 1996). Supporting that TEOAEs may not be object to prenatal masculinization, are that the TEOAEs in neonates were recorded a few days after birth, and in a larger subject group as the young adult twins. Twin testosterone transfer has been shown in studies of litter bearing rodents (Clemens et al., 1978; Kinsley et al., 1986; vom Saal & Bronson, 1980), where females surrounded by males in the uterus demonstrated frequent and uncharacteristic mounting behavior (Clemens et al., 1978). Contrary to expectation, females in OS twin pairs showed a mean TEOAE level that was significantly larger (2.1 dB) compared to male co-twins, and numerically larger TEOAEs than female non-twins and females in SS twin pairs (Figure 13).

6.3 Etiology and auditory profiles in infants with congenital uSNHL

6.3.1 Pure tone thresholds (PTTs) in congenital uSNHL (Study I, III, and IV)

The 20 consecutively recruited infants with uSNHL from the universal NHS program in Stockholm showed a median ABRthr of 55 dB nHL in their IEs (n = 20), while those with recordable ABRthr showed a median of 43 dB nHL (n = 12). In study I the median (and the mean) PTA was 45 dB HL (n = 6), where the shape of the PTT versus frequency functions (i.e., audiograms) varied greatly (Figure 8).

The median 55 dB nHL in the IE of infants with congenital uSNHL (n = 20) were similar to previous results of neonates with congenital uSNHL measured in Region Stockholm showing 50 dB nHL median in their IEs (n = 18) (Berninger & Westling, 2011).

Measurement type and dB scales need to be taken into consideration in the comparison of hearing thresholds in study I compared to studies III and IV. An ABRthr of 20 dB nHL in infants below approximately the age of 6 months corresponds roughly to an adult threshold of about 30 dB HL due to the acoustics of the smaller ear (Berninger & Westling, 2011; Marcoux & Hansen, 2003; Sininger & Abdala, 1996). The ABRthr measured with clicks corresponds best to a behavioral dB HL threshold in the 1-4 kHz region (Roeser et al., 2007). The median threshold based on the 1, 2, 3, 4 kHz average in study I was 49 dB HL, corresponding to an ABRthr in neonates of about 39 dB nHL (Johansson et al., 2020a) (n = 6). Thus, the estimated 39 dB nHL ABRthr was numerically lower, but not by much compared to the median ABRthr in study III of 43 dB nHL for aidable uSNHL (n = 12).

6.3.2 Malformations in congenital uSNHL (Study III)

Nine out of the 14 infants that agreed to participate in MRI (19 eligible) showed a malformation (i.e., 64%, Table 4). Malformations were also common for all degrees of uSNHL; 43% in mild to severe uSNHL (3/7), and 86% in SSD (6/7).

The 64% malformations in congenital uSNHL were numerically larger than previous studies of malformations in uSNHL found by MRI of 37-58% (Clemmens et al., 2013; Gruber et al., 2017; Orzan et al., 2021; Paul et al., 2017; van Beeck Calkoen et al., 2019). The congenital origin of the uSNHL may largely explain the numerically higher percentage, as 64% is closer to the 46-67% prevalence of malformations when acquired uSNHL was excluded, as shown both with MRI (Orzan et al., 2021) and CT (Masuda et al., 2013; Nakano et al., 2013; Orzan et al., 2021). MRI and CT show malformations in somewhat different ways, even if both methods have shown efficient in diagnosing malformations in uSNHL (Clemmens et al., 2013; Gruber et al., 2017; Johansson et al., 2022; Masuda et al., 2013; Nakano et al., 2013; Orzan et al., 2021; Paul et al., 2017; van Beeck Calkoen et al., 2017). With MRI the vestibulocochlear nerve can be observed, as soft-tissue resolution is higher, whereas CT provides a better view of the bony labyrinth and middle-ear. With CT cochlear aplasia or hypoplasia is mainly diagnosed due to abnormalities in the bony cochlear nerve canal or internal auditory canal (Nakano et al., 2013; Orzan et al., 2021).

Half of the MRI scans demonstrated cochlear nerve aplasia or hypoplasia (Figure 14), which was numerically higher than the 17-36% in previous studies of uSNHL (Clemmens et al., 2013; Gruber et al., 2017; Orzan et al., 2021; Paul et al., 2017; van Beeck Calkoen et al., 2017). The numerically higher percentage may be due to the congenital origin of the uSNHL in study I, as the first prospective study of a larger group of children with uSNHL in which all were identified and diagnosed shortly after birth (Johansson et al., 2022). One previous retrospective study of SSD found that the incidence for cochlear nerve aplasia or hypoplasia was 100% in infants (n = 10), 75% in preschool children (n = 20), and 48% in children with SSD in general (n = 50), indicating that the diagnosis age matters (Clemmens et al., 2013). The numerically higher percentage may also be due to the MRI resolution, as a 3T scanner was used in 93% of our scans. However, it is difficult to assess as most previous studies of uSNHL did not report the MRI resolution (Gruber et al., 2017; Paul et al., 2017; van Beeck Calkoen et al., 2017), but one study reported using a 1.5T resolution (Orzan et al., 2021), and another a 3T resolution (Clemmens et al., 2013).

One out of seven infants (14%) with mild-to severe uSNHL showed cochlear nerve aplasia or hypoplasia, whereas the prevalence was much higher for SSD, as expected (6/7 infants, i.e., 86%).

The 4 out of 14 inner ear malformations (29%) was similar to the 28-46% previously found in children with uSNHL (Clemmens et al., 2013; Orzan et al., 2021; Paul et al., 2017; van Beeck Calkoen et al., 2017).

The 3 out of 14 subjects with EVA (21%) was also similar to previous studies of 8-25% (Clemmens et al., 2013; Orzan et al., 2021; Paul et al., 2017; van Beeck Calkoen et al., 2017). One of the subjects had bilateral EVAs and hearing quickly deteriorated to a bilateral SNHL. The bilateral EVAs were found with MRI at 7 months, and HL progression was found

bilaterally at hearing test follow up at 8 months (TEOAEs in NE no longer normal, ABRthr in both ears worse) (Johansson et al., 2022). Subsequently, Pendred syndrome was diagnosed (Johansson et al., 2023), and the child was fitted with a CI, followed by another CI when the HL progressed further later in development, motivating the use of MRI early in development.

Malformations were common for all degrees of uSNHL, in agreement with a few previous retrospective studies of uSNHL (McClay et al., 2008; van Beeck Calkoen et al., 2017). Malformations were about twice as common for SSD compared to mild to severe uSNHL in study III, also similar to the findings of McClay et al. (2008).

6.3.3 Congenital CMV infection—an uncommon cause for congenital uSNHL? (Study III)

Contrary to expectations, none of the infants with congenital uSNHL were positive for cCMV infection. Previous studies of uSNHL found cCMV infection to be a cause of HL for about 10% (Paul et al., 2017) to 20% (Arndt et al., 2015; Karltorp et al., 2012) of subjects. The most likely explanation of the absence of cCMV infection is probably the congenital onset of uSNHL and our prospective study design, although the Covid-19 pandemic during the recruitment years may also have contributed to the result.

It is not surprising that cCMV infection is less prevalent in congenital uSNHL compared to uSNHL in general, as the SNHL associated with cCMV is often progressive (Fowler et al., 1997). Moreover, most children with cCMV associated SNHL appear to develop the HL after the neonatal period (Fowler et al., 1999; Fowler et al., 2017). These studies defined the neonatal period from birth to 2 months, whereas uSNHL in study III was already identified around postnatal day 3. Thus, with our strict inclusion criteria for congenital uSNHL an even larger amount of uSNHL with cCMV infection cause would not have been included in study III compared to the neonatal period group in the previous studies of SNHL (Fowler et al., 1999; Fowler et al., 2017).

It has been known for a long time that cCMV infection spread varies over time, and the Covid-19-pandemic may have contributed to the lower spread. In countries with strict lockdowns the cCMV infection prevalence decreased (Fernandez et al., 2022; Rios-Barnes et al., 2022). Although Sweden was not a country under strict lockdown, adjustments in hand hygiene were made, and close contact with other people was avoided for a long period of time. More importantly perhaps for infection spread in families were stricter rules for attendance at kindergarten with regards to cold symptoms, thus limiting spread of potential cCMV to soon-to-be older siblings, as well as stricter rules regarding hospital visits with cold symptoms. Many prenatal gatherings and courses were cancelled or moved to online forums instead of in person.

The type of cCMV infection test may of course also have contributed to the discrepancy in cCMV infection prevalence. However, the DBS cards with CMV PCR technique was also used in the study by Karltorp et al. (2012) that showed a prevalence of 20% in uSNHL. The CMV PCR technique has also shown a high negative predictive value of 0.99 (95% CI = 0.972-0.997), as found by a meta-analysis (Wang et al., 2015), although the sensitivity of the test has been questioned as compared to saliva rapid culture tests taken shortly after birth (Boppana et al., 2010).

6.3.4 Genetic causes for congenital uSNHL (Study IV)

In all the three infants with a co-morbidity observed at birth a genetic cause for the uSNHL could be established (3/3 infants), compared to 2/15 of the remaining infants with congenital uSNHL.

In total, 28% of congenital uSNHL were from genetic causes (5/18), compared to the large spread of 6-43% in previous studies of uSNHL (Gruber et al., 2017; Haffey et al., 2013; Paul et al., 2017; van Beeck Calkoen et al., 2019). Our percentage of genetic causes may be higher than some other studies because of the much later age at diagnosis reported in previous studies of 3.3-7 years of age (Gruber et al., 2017; Haffey et al., 2013; Paul et al., 2017; van Beeck Calkoen et al., 2019), compared to 2 months in study IV. Our percentage of genetic causes may alternatively be lower than some other studies because of previous retrospective designs with risks of overestimation of genetic causes because of selective sampling (mainly children with suspected genetic causes may have been advised genetic testing). Furthermore, our study tested for a larger number of genetic mutations than previous studies. The OtoSCOPE® v.9 is the genetic test panel screening for the largest number of mutations so far (224 gene mutations, 15 infants), and the other test panels used were also broad (105-137 gene mutations or chromosomal defects, 4 infants). One study used OtoSCOPE® v.4 and v.5 screening for 66-89 gene mutations and found only 1% genetic causes for uSNHL (1/69 subjects), and 3% for congenital uSNHL (1/35 subjects) (Sloan-Heggen et al., 2016), indicating that the number of mutations screened almost certainly matters.

One of the subjects had two mutations in the GJB2 gene, associated with autosomal recessive non-syndromic SNHL, often referred to by the name of the coding protein: Connexin-26 (subject 2, Table 4). According to previous studies, the GJB2 gene appears to be the gene associated with most genetic causes for uSNHL, although with a large spread in incidence of 0-31% in uSNHL (Haffey et al., 2013; Lee et al., 2009; Paul et al., 2017; Sloan-Heggen et al., 2016; van Beeck Calkoen et al., 2019). The study that found zero cases of GJB2 in 69 individuals with uSNHL also questioned if it can be associated with uSNHL at all, or if it always affects both ears (Sloan-Heggen et al., 2016). Our finding supports that congenital uSNHL is associated with GJB2 associated non-syndromic uSNHL, due to the NE TEOAEs and ABRthr of ≤20 dB nHL in the NE in subject 2, at two months of age.

However, as an infant's click ABRthr of ≤20 dB nHL is approximately equivalent to an adult PTT of ≤30 dB HL (Marcoux & Hansen, 2003; Sininger & Abdala, 1996), and the ABRthr in the other ear was not so much higher at 35 dB nHL, an asymmetric SNHL cannot be fully excluded. The GJB2-mutation is also a common mutation in general, with an incidence of about 1 in 2500 births (Morton & Nance, 2006).

Pendred syndrome diagnosed in subject 7 is less common in newborns, with an incidence of 1 in 10 000 to 20 000 births (Morton & Nance, 2006; Sloan-Heggen et al., 2016). Pendred syndrome has, like the GJB2-mutation, mostly been associated with bilateral SNHL (Sloan-Heggen et al., 2016; van Beeck Calkoen et al., 2019). It is also associated with bilateral EVAs, as for subject 7, and not with unilateral EVAs (Greinwald et al., 2013; Pryor et al., 2005). How prevalent Pedred syndrome is in uSNHL is still unknown, although a meta-analysis estimated the incidence of bilateral EVAs in uSNHL to be 2% (Ropers et al., 2019) (as compared to 5% in study IV). However, due to the SNHL progression associated with EVAs, and the diagnostic age of >4 years in the previous studies (Colvin et al., 2006; Greinwald et al., 2013; Ropers et al., 2019), the 2% is probably an underestimation.

Children with CHARGE syndrome, as diagnosed in subject 19, may also show bilateral SNHL, and has a similar incidence in newborns as Pendred syndrome at 1 in 10 000 births (Sanlaville & Verloes, 2007). CHARGE syndrome has also been found in previous studies of uSNHL (Haffey et al., 2013; van Beeck Calkoen et al., 2019). The incidence in uSNHL is difficult to estimate due to one child in each study; 1 in 14 (7% (Haffey et al., 2013), 1 in 57 (2% (van Beeck Calkoen et al., 2019), and 1 in 20 in study IV (5%).

The 8p inverted duplication and deletion syndrome in subject 10 (incidence 1 in 10 000-300 000 births (García-Santiago et al., 2015)) and Townes-Brocks syndrome (incidence estimated to 1 in 250 000 births (Martínez-Frías et al., 1999)) in subject 3 have not, as far as I know, been reported in studies on uSNHL previously.

Previous studies have also connected other single cases of syndromes/genetic mutations to uSNHL: VACTERL syndrome (Haffey et al., 2013), Goldenhar syndrome (Haffey et al., 2013), Branchiootorenal (BOR) syndrome, and inversion and deletion in the 13q32-34 region (Paul et al., 2017).

Waardenburg syndrome has also been diagnosed in two single cases in two separate studies of children with uSNHL (Haffey et al., 2013; Usami et al., 2017), and was also suspected in another study due to clear characteristics of the syndrome in four subjects (Paul et al., 2017). Waardenburg syndrome type 2 was discussed as a cause for subject 13, but as none of the characteristics of the syndrome was present, and the KITLG gene is of uncertain significance in the syndrome, it could not be established as a cause for the uSNHL (Johansson et al., 2023). A lot of genetic variants were found with the OtoSCOPE® v.9 genetic panel, in total 198 variants (Figure 15). Of these, 2% were catheterized as pathogenic, 1% as likely pathogenic, and 60% of uncertain significance. The large number

of cases of uncertain significance indicate the importance of expertise in the genetic diagnosis of uSNHL and the importance of supportive clinical findings in the determination of causality. One variant for subject 18 was categorized as pathogenic, but in closer inspection only in a homozygous state (TMC1 pathogenic for autosomal recessive non-syndromic HL at the DFNB11 locus), indicating it was not as cause for the uSNHL as subject 18's variant was in a heterozygous state. Another variant for subject 15 was categorized as likely pathogenic, but with missense allele, and the allele for subject 15 was a null allele. Thus, it was not likely the cause for the uSNHL.

A genetic cause for the uSNHL was found in two out of three subjects diagnosed with EVA (subject 3, Townes-Brocks syndrome and subject 7, Pendred syndrome), indicating that it is probably a good indicator for performing a genetic test. Other syndromes have also been associated with EVA that have also been found as causes for uSNHL including BOR, CHARGE, and Waardenburg syndromes (Pryor et al., 2005).

Four out of five subjects with a genetic cause for the uSNHL showed a mild to moderate degree of uSNHL (35-45 dB nHL in the IE), corroborating one other study that found mostly mild to moderate degree of uSNHL to be associated with a genetic cause (Gruber et al., 2017).

6.4 Strength and limitations

A strength with all included studies were the objective measurements used, including measurements of TEOAEs, DPOAEs, ABRs, ARTs, tympanograms, SLA, and RECDs with coupler verification (Asp et al., 2016; Bagatto et al., 2005; Gelfand, 2016; Kemp et al., 1990; Roeser et al., 2007). The measurements were performed in controlled sound environments; the tympanograms, ARTs, and RECDs and coupler measurements in a quiet room or in an audiometric test room, whereas the TEOAEs, DPOAEs, SLAs, ABRs, SRTs in competing speech and the Bekesy audiometry were always performed in a audiometric test room allowing threshold detections down to -10 dB HL (ISO8253-1, 2010).

The same tester who was part of our research group also performed all measurements in studies I, III and IV. The doctoral student performed the measurements in study I, except for SLA where Dr Filip Asp performed most of the measurements, and ABR where Associate Professor Berninger performed the measurements together with the doctoral student. In the studies III and IV clinical audiologist Maria Drott performed the measurements together with the doctoral student, and at some research visits assisted by Associate Professor Berninger. The determination of the ABR waves was also performed by Associate Professor Berninger who was blinded to which ear was under analysis in studies (with the assistance of the doctoral student).

Another strength was the use of the same test equipment and procedure for all subjects in all studies (for tympanometry, ARTs, ABR, PTTs, SLA, SRTs in competing speech, APHAB,

PEACH, TEOAEs, DPOAEs, and SECDI III). The only exception being 1) the RECD measurement for one subject's RECD in study I, 2) the MRI equipment where three different, but similar, equipment were used, 3) genetic testing where most subjects were measured with the OTOscope® v.9. However, four subjects also/instead were tested with Karolinska University Laboratory's targeted gene panels, and 4) cCMV testing where most infants were tested with the DBS card after the mother was CMV IgG or IgM positive. However, three mothers were both IgG and IgM negative and the infant did not need cCMV testing. Moreover, one subject had already been tested with plasma DNA the same day as birth.

The SLA and SRT measurements included test and re-test measurements to evaluate reliability and were used in several previous studies (Asp et al., 2018; Asp et al., 2016; Berninger & Karlsson, 1999). Specifically, one study evaluated the effect of simulated uHL, which indicated a high sensitivity of the tests in detecting significant differences in small research groups (i.e., n = 8) under similar subject conditions (Asp et al., 2018). ABRs, TEOAEs, DPOAEs, and tympanograms are well-known audiologic tests that have been used in numerous previous research studies in various research groups (Roeser et al., 2007). The APHAB and PEACH has been used in several previous studies in similar age-groups for easy comparison to children with NH (Bagatto & Scollie, 2013; Brännström et al., 2014; Rance et al., 2014) and has also been found to be effective in aided vs. unaided comparisons (Bagatto et al., 2016; Ching et al., 2018; Kopun & Stelmachowicz, 1998; Rance et al., 2014), and for unaided uSNHL performance in younger age-groups (Fitzpatrick et al., 2019). The analysis of the MRI scans also included a first analysis by one or two experienced neuroradiologists or head-neck radiologists, and a re-analysis of an experienced heck-neck radiologist before publication of the results.

RECD and coupler measurements were used to quantify HA amplification and ensure HAs that were functional, which has not been quantified in previous HA studies in uHL (Benchetrit et al., 2022; Briggs et al., 2011; Johnstone et al., 2010; Rohlfs et al., 2017; Updike, 1994). Nevertheless, Benchetrit et al. (2022) that only included questionnaires as HA outcomes measured real ear measurements and verified the HAs using the Audioscan Verifit 1. Although no quantifiable results of the measurements were presented, the HAs were reported to be "matched to Desired Sensation Level v 5.0 child targets as closely as possible".

Other strengths in the study design included careful exclusion of outer and middle ear disorders by the used of otomicroscopy by an experienced otologist, tympanometry, ART and ABR measurements (studies I, III and IV). In the inclusion of subjects to studies III and IV the repeated measurements of TEOAE, aABR and ABR over time also contributed to effects of, e.g., temporary OME to resolve on its own.

Studies I, III and IV also used comprehensive test batteries to answer the research questions. In studies III and IV a strength in the study design was the auditory profiles describing the characteristics of congenital uSNHL in detail, which is sparsely described in previous studies of uSNHL and etiology. In studies I, III and IV the strict criterion for the NE is also a strength of the studies: ≤20 dB HL (0.25-8 kHz) in study I and ≤20 dB nHL in studies III and IV.

The reason we decided to focus on congenital uSNHL is that one fourth of the newborns found with SNHL in the NHS programs have congenital uSNHL. Congenital uSNHL can be diagnosed very early in development, and results directly implemented into clinical practice. A comparison to children with acquired uSNHL could of course also be of large value but need to include about twice as many subjects, due to the differences in etiology between the groups. Even in congenital uSNHL the thesis shows results that the degree of uSNHL varies largely, and so do the configurations of the audiograms, and the cause for uSNHL (Figure 8, and Table 4).

Study II had the strength of the largest group of twins studied with TEOAEs to date (n = 454), and a very large non-twin group (n = 21 199). Studies I, III and IV had statistically rather small sample sizes, especially study I (n = 6 subjects). However, previous studies of HA outcomes in children with uHL has been similar in size: n = 8 (Briggs et al., 2011), n = 6+6 (two age groups) (Johnstone et al., 2010), n = 6 (Updike, 1994). We also invited all children with congenital uSNHL 6-11 years of age, with more than 6 months of HA use in Region Stockholm, and only one eligible subject declined participation. Thus, the few subjects also reflect the lack of effective HA interventions in the group.

A recent study included a larger study group of 34-37 children with uHL that had not received HA intervention (three did not complete testing) in a HA trial of questionnaire outcomes (Benchetrit et al., 2022). Nevertheless, 62% had an acquired uHL and 59% had conductive uHL, indicating very different perceptual consequences and etiology of the uHL compared to the children in study I, with no subgrouping according to these parameters in the results. Benchetrit et al. (2022) concluded with: "Further research discerning which subgroups of children with UHL would benefit most from the addition of a HA to baseline accommodations is warranted". The small group of six children in study I is a limitation, even if the strict inclusion criteria for congenital uSNHL probably made the sample representative of the population, despite its small size. Nonetheless, if relevant statistically significant effects are recorded in a small representative group, they are likely worth further investigation. The group of 20 infants with congenital uSNHL in studies III and IV were also similar in size to that of previous studies of uSNHL, where children with congenital uSNHL specifically has not been under etiology evaluation with a prospective study design, to my knowledge. However, it was unfortunate that 5 out of 20 infants with families declined MRI testing. Yet, due to the high diagnostic yield, the study still contributes significantly to the research field, especially due to the comparably

homogenous group of infants with congenital uSNHL where all were invited to MRI and the prospective study design.

The largest strength with studies III and IV was probably the prospective study design, with an aim of inviting all children born with uSNHL in Region Stockholm during a two-year period. All previous studies I am aware of are either retrospective studies, typically medical chart reviews, or children with uSNHL that were referred to etiologic testing, without referring all infants with uSNHL, only selected cases, e.g., (Gruber et al., 2017; Haffey et al., 2013; Paul et al., 2017; Sloan-Heggen et al., 2016; Tropitzsch et al., 2022; van Beeck Calkoen et al., 2019; van Beeck Calkoen et al., 2017). It should be noted that the results of etiology in studies III and IV can mostly be generalizable to developed countries, due to, e.g., infection exposure and health during pregnancy under considerably different living standards. Genetic syndromes are also known to vary between countries, although it is noteworthy that 45% of the 20 families of infants in studies III and IV spoke only Swedish in the homes, indicating that most families presumably also had backgrounds and/or relations from non-Swedish countries.

Another strength in studies III and IV was the early diagnosis age of congenital uSNHL in studies III and IV, e.g., a median of 2.2 months of age in comparison to more than 4 years in previous MRI studies of uSNHL (Masuda et al., 2013; Nakano et al., 2013; Orzan et al., 2021), where acquired uSNHL was excluded, and genetic outcome studies in uSNHL (Gruber et al., 2017; Paul et al., 2017; van Beeck Calkoen et al., 2019). The early identification age of HL/no HL with TEOAEs a few days after birth was a strength in all studies in the thesis.

Study IV also had the strength of using the broadest test panel used for genetic testing and analysis of uSNHL in children so far. It should be noted that whole-genome sequencing has been more easily available lately due to decrease in costs and may be a good alternative to broad test panels clinically in the future. However, the focus should probably still be on the genes known to be associated with uSNHL. Ethical concerns need careful consideration, e.g., what can be discovered in addition to HL causes. The significant difference in findings based on non-syndromic vs alleged syndromic uSNHL has not been demonstrated before; nevertheless, an expert group has previously formulated recommendations for genetic testing of suspected syndromic uSNHL (Liming et al., 2016).

A limitation in study I was the large variability in HA usage according to datalogging. PEACH subjective opinion regarding HA use filled in by the parents were used to confirm the datalogging's accuracy and showed similar trends in use. The variability would have been interesting in a larger sample, but as no significant effects could be attributed to HA usage time based on datalogging, the heterogeneity was not optimal. Yet, the HA usage time could not be changed based on the study design, because we wanted children to use their HAs as they do in everyday life, and HA usage time varied in congenital uSNHL.

It was also difficult to assess the effect of HA parameters in study I. All used adaptive directional microphones, that may not have been optimal for SLA, and noise reduction. Yet, similar settings for all subjects are rather a benefit in a small group. Amplification settings were, however, different within the group. Based on the findings of study III, children with congenital uSNHL may not have recruitment, suggesting that it may not make a difference if the children used the Desired sensation level (DSL) v.5 or National acoustic laboratories Non-linear 1 (NAL-NL1) or Widex own prescription method. Rather, it was more important to distinguish weather the prescription method setting included wide dynamic range compression or a setting closer to linear amplification with limiting. The children with uSNHL demonstrated high aided SII values, which was a strength in the study (Johansson et al., 2020a), but we did not evaluate the compression setting further.

The Covid-19 pandemic was an unexpected factor to consider in studies III and IV. I believe it mainly affected the cCMV infection spread during the study years of 2020, which would partly explain our lack of infants with congenital uSNHL and cCMV infection, a trend observed in countries with strict lockdowns (Fernandez et al., 2022; Rios-Barnes et al., 2022). However, the cCMV infection can only cause uSNHL in utero, and the spread of the Covid-19 pandemic first started in March 2020. Thus, the majority of mothers included, expecting a baby in 2019 and the first part of 2020 was not affected by the pandemic.

7 Conclusions

General conclusions about congenital uSNHL:

- Malformations of the auditory system are common for all degrees of uSNHL, and especially for profound uSNHL (i.e., SSD).
- Genetic causes are common in children with co-morbidities observed at birth, while most alleged non-syndromic causes remains unknown.
- Genetic causes include autosomal recessive non-syndromic SNHL (GJB2/Connexin-26), CHARGE syndrome, Pendred syndrome, Waardeburg syndrome, Townes-Brocks syndrome, VACTERL syndrome, Goldenhar syndrome, BOR syndrome, chromosome 8P inverted duplication and deletion syndrome, and inversion and deletion in the 13q32–34 region.
- Congenital uSNHL varies from mild to profound degree, with a median about 50-55 dB nHL in neonates, corresponding roughly to 40-45 dB HL in adults with uSNHL. The configuration of the PTT by frequency functions (i.e., audiograms) show large variability in shape.

School-aged children (10-11 years old) with congenital uSNHL:

- Struggle with communication in demanding listening environments with and without HAs, based on both questionnaire and psychoacoustic test results.
- Benefit from HAs in one-to-one communication, based on child and parent questionnaires, despite late HA fittings (after 5 years of age). We therefore recommend offering a HA trial, and family-centered counselling should offer realistic expectations , given the limited benefit in difficult listening environments and in SLA. Outcomes should preferably be measured of individual HA benefit.
- Have significant HA dis-benefit in SLA with late fitted HAs on a group level, although between-individual variation exists.
- May gain more benefit from HAs fitted early, as a close relationship between aided SLA and the ABR I-V interval was found in children with congenital uSNHL fitted late, indicating that a longer transmission time to the upper brainstem (less mature and less typical transmission) may result in poorer aided SLA.

Infants with congenital uSNHL:

- Demonstrate a large malformation prevalence of 64% (9/14 MRI scans), for all degrees of uSNHL. In ears with profound uSNHL the malformation prevalence was especially high at 86% (6/7 MRI scans), although also high at 43% for mild to severe uSNHL (3/7 MRI scans).
- Showed the highest malformation prevalence for cochlear nerve aplasia or hypoplasia found in 50% of the 14 MRI scans, followed by inner ear malformations observed in 29% of the MRI scans. Of all MRI scans 21% demonstrated EVA.

- May have a low incidence of cCMV infection, as none of the 20 infants with congenital uSNHL showed a positive cCMV infection test. However, the influence of the Covid-19 pandemic and fluctuations in infection spread over time may have lead to a lower than usual incidence during the study years.
- Showed a genetic cause for the congential uSNHL in 28% of genetic results (5/18 infants with congenital uSNHL). All three infants with co-morbidities observed at birth received a gentic diagnosis: CHARGE syndrome (CHD7), Townes–Brocks syndrome (SALL1), and Chromosome 8P inverted duplication and deletion syndrome. One infant with bilateral EVA found with MRI was diagnosed with Pendred Syndrome (SLC26A4). The last subject was diagnosed with autosomal recessive non-syndromic HL (GJB2/Connexin 26).
- Demonstrated parallell shifted IE and NE ABR I/O functions, indicating that most infants with congenital uSNHL did not experience the typical loudness recruitment associated with cochlear SNHL.

Neonatal TEOAEs:

- Show up to 100% heritability, based on neonatal twin heritability estimates, despite large between-individual variability.
- Measures in twins did not support the twin testosterone transfer hypothesis, as neonatal twins in OS twin pairs did not show masculinized TEOAE levels, as has previously been observed based on OAEs of young adult twins.

8 Points of perspective

8.1 Clinical implications

8.1.1 Etiology investigation

In studies III and IV we found that MRI is powerful in diagnosing malformations in congenital uSNHL, and that genetic testing is efficient in diagnosing alleged syndromic congenital uSNHL in infants. Even if only three infants showed co-morbidities at birth, all were diagnosed with a genetic cause of uSNHL. It was also statistically significantly more likely for the infants with alleged syndromic uSNHL at birth to be diagnosed with a genetic cause for uSNHL compared to infants with alleged non-syndromic uSNHL. Our results support a previous research opinion recommending genetic testing for suspected syndromes in uSNHL (Liming et al., 2016), although I am not aware of any published studies showing a high rate of genetic causes in congenital uSNHL with suspected syndromes previously.

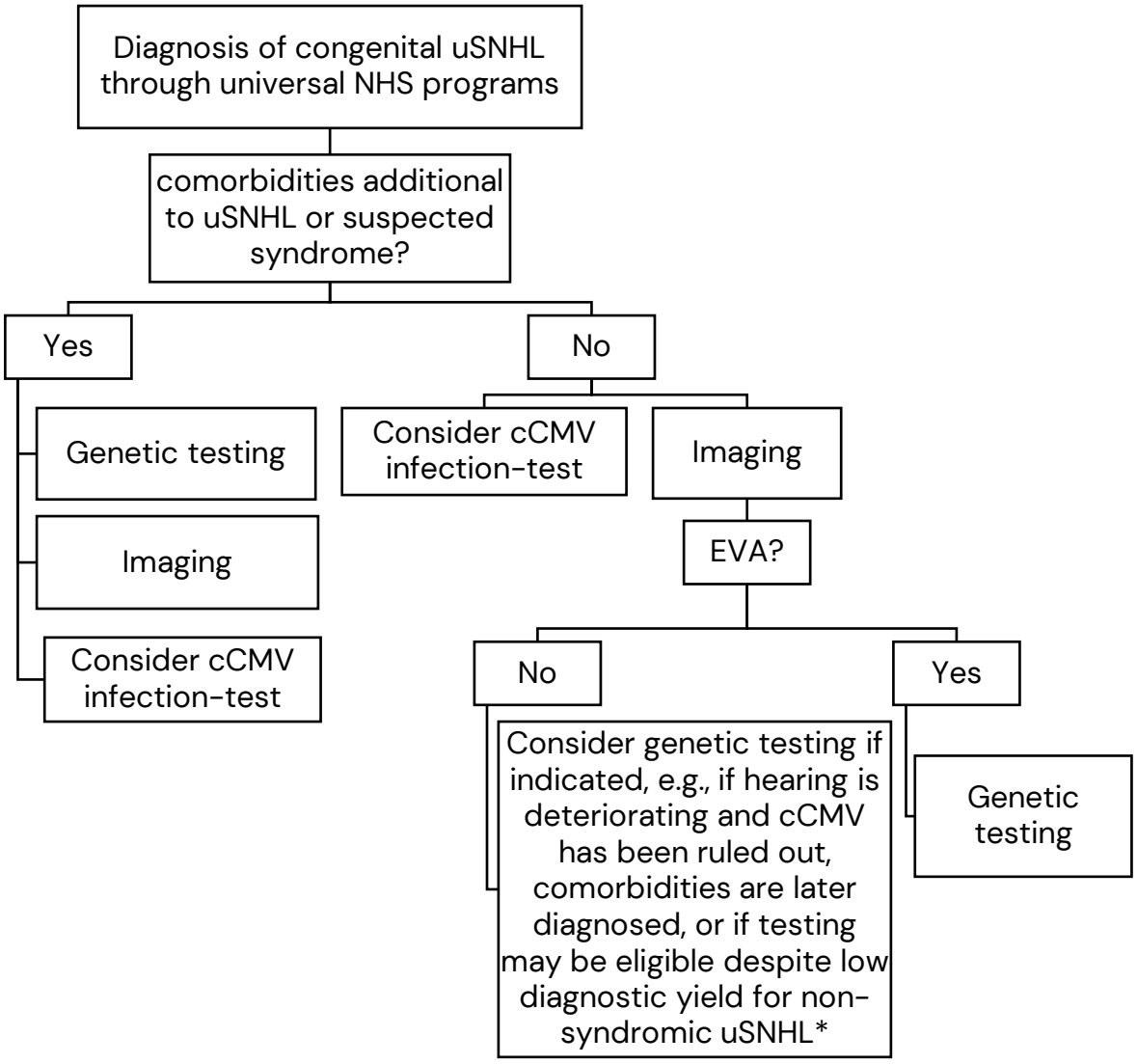

Figure 17. Recommendation for diagnostic work-up of children with congenital uSNHL based on studies III and IV together with previous research in uSNHL. *Genetic causes for non-syndromic uSNHL were found in 7% of infants with alleged non-syndromic congenital uSNHL in study IV (1 out of 14 subjects with no syndrome diagnosis, the remaining 4 subjects revealed syndromes summing up to 18 subjects genetically tested in total). cCMV = congenital cytomegalovirus; EVA = enlarged vestibular aqueduct; NHS = newborn hearing screening; uSNHL = unilateral sensorineural hearing loss. Adapted version of Figure 1 by Johansson et al. (2023), published open access by MDPI.

Based on the findings, in combination with findings from previous research in children with uSNHL in general, we recommend imaging to be part of the etiologic evaluation of children with congenital uSNHL (Figure 17). MRI may be the first choice if the congenital uSNHL is diagnosed early, as CT is not possible during the first years of life due to radiation. Both CT (Masuda et al., 2013; Nakano et al., 2013; van Beeck Calkoen et al., 2017), and MRI (Clemmens et al., 2013; Gruber et al., 2017; Johansson et al., 2022; Orzan et al., 2021; Paul et al., 2017; van Beeck Calkoen et al., 2017) has shown to be effective in diagnosing malformations in congenital uSNHL despite their method differences.

We recommend genetic testing for suspected syndromes (Figure 17). The combined diagnostic yield of MRI and genetic testing was 71% (10/14 subjects) and may appear similar to the 64% diagnostic yield for only MRI (9/14 subjects). Yet, MRI is largely diagnosing site of lesion, while genetic testing is diagnosing the primary cause for the congenital uSNHL and may add important information in how to support the child with congenital uSNHL. For example, in the subject with bilateral EVAs the diagnosis of Pendred syndrome may help in risk assessment of progression of SNHL, and the thyroid gland is often affected.

A syndrome may be suspected due to co-morbidities, as for the three subjects with a genetic diagnosis and malformations observed at birth in study IV. A syndrome may also be suspected based on MRI-findings (Figure 17). We recommend genetic testing for EVAs, as several syndromes have been diagnosed in combination with unilateral and bilateral EVAs including Pendred (bilateral EVAs), BOR, CHARGE, Townes-Brocks and Waardenburg syndromes (Johansson et al., 2023; Pryor et al., 2005). In study IV we found that two out of three infants with EVA were diagnosed with a genetic cause for the congenital uSNHL. It can still be argued that not enough evidence exists that indicates that genetic testing in EVA is efficient, but based on the associated syndromes, we suggest this recommendation until the contrary is established.

A clinical recommendation regarding cCMV infection in congenital uSNHL is more difficult to establish. Based on my colleagues and my clinical experience, cCMV infections are diagnosed in children with uSNHL sometimes found in NHS programs. However, the HL may progress very rapidly to a bilateral SNHL, like in one potential study subject who seemed eligible based on the NHS results and first clinical ABR, but showed bilateral SNHL at the research visit at 2 months of age when final inclusion and exclusion criteria were

applied. The infant received bilateral CIs shortly afterwards. However, based on the 20 infants with established congenital uSNHL based on the inclusion and exclusion criteria of study IV, none showed congenital cCMV infection. Nonetheless, the Covid-19 pandemic and less infection spread over time should be taken into consideration. An argument for cCMV infection testing is the relatively low testing and participation costs, and the risks of HL progression (Fowler et al., 1997; Vos et al., 2021) that may warrant CI intervention (Arndt et al., 2015; Arras et al., 2022; Polonenko et al., 2017a; Thomas et al., 2017). On the other hand, the prevalence of cCMV infection in congenital uSNHL should be considered low, despite 6-20% prevalence found in a few studies of uSNHL in general (Karltorp et al., 2012; Paul et al., 2017; Usami et al., 2017), it has also been found that most children with uSNHL and cCMV infection develop uSNHL after the NHS period (Fowler et al., 1999; Fowler et al., 2017).

A clinical recommendation regarding genetic testing for alleged non-syndromic uSNHL with no EVA found with imaging is also challenging, based on current knowledge. Excluding the syndromic patients (three diagnosed based on co-morbidities, and one from bilateral EVAs with imaging), 1 out of 14 infants (7%) with alleged non-syndromic uSNHL was diagnosed with a genetic diagnosis: non-syndromic HL caused by the GJB2 gene coding for Connexin 26. If 7% of children with a non-syndromic have a genetic cause for uSNHL, genetic testing could be considered motivated, especially if it could enable better clinical intervention for the infant. However, the risk for HL progression related to Connexin 26 is low, as approximately 90% are stable over time (Cama et al., 2009; Orzan et al., 1999). Thus, we suggest clinical testing for genetic causes in alleged non-syndromic uSNHL mainly for the following set of conditions: 1) EVA; 2) the HL is deteriorating and a cCMV infection cause has been ruled out; 3) a co-morbidity, pigmentation, new knowledge about family history of HL or other indication for a syndromic HL that may not have been detected shortly after birth is revealed; 4) A family may also want to know the cause for the uSNHL for various reasons, and be able to afford the cost for testing, even if the hospital may have decided it is not part of the clinical protocol based on the relatively low diagnostic yield (Figure 17).

Our results also suggest that it may be a good idea to continue genetic testing based on assumed phenotypes in infants with co-morbidities, especially if the first test is a chromosomal array, like for subject 19, that was diagnosed with CHARGE syndrome with the OtoSCOPE® v.9 panel as part of study IV.

8.1.2 HA intervention in congenital uSNHL

Primarily research is needed for early HA fittings in congenital uSNHL. Based on the subjective HA benefit found in one-to-one communication for HA fittings after five years of age in study I, and previous HA benefit found based on questionnaires in previous studies of uHL fitted after six years of age (Benchetrit et al., 2022; Briggs et al., 2011) we

suggest HA intervention for children with mild to moderate congenital uSNHL. However, expectations regarding the HA trial should be managed through family-centered counselling, given the limited benefit in difficult listening situations and potential dis-benefit in SLA. Optimally, individual HA benefit over time should also be measured with various testing methods, and HA function and adequate amplification verified.

8.2 Future directions for research in congenital uSNHL

8.2.1 HA outcomes

Results from study I and Johnstone et al. (2010) indicate that early HA fittings, compared to later HA fittings, when the brain is more adaptive, may result in better aided SLA outcomes. Improvements in SLA in turn may help the children with uSNHL in more difficult listening environments where both ears are needed, and no psychoacoustic test in children with uSNHL using HAs has shown aided benefit as of yet (Briggs et al., 2011; Johansson et al., 2020a). No studies have so far looked at HA fittings introduced to uSNHL before 4 years of age, which would be of value to the research field. Thus, it was part of the PhD project to follow the hearing development and speech-language development of the children with congenital uSNHL included in studies III and IV, from diagnosis every half-a-year until the child was 2.5 years of age (Figure 18). However, time was a constraint, and we thought the PhD thesis comprehensive enough without including the longitudinal outcomes. The longitudinal test battery included measurements of TEOAEs, ABRs, tympanograms, ARTs, SLA (with and without HA), and visual reinforcement audiometry. Three questionnaires or word inventory lists were also filled in at each visit: PEACH, The LittlEARs® auditory questionnaire, and SECDI I, II and/or III.

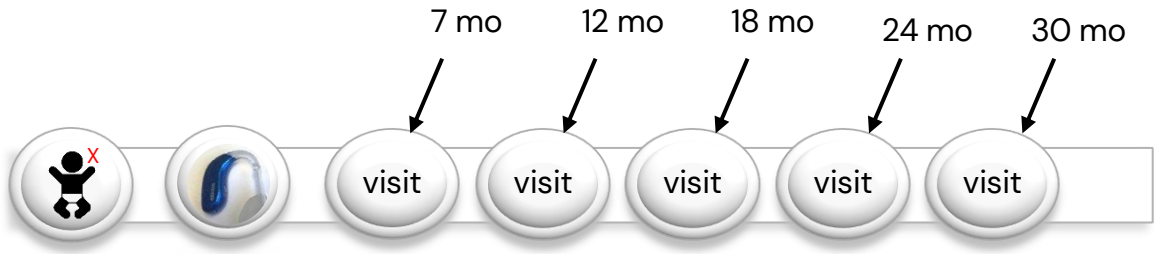

Figure 18. Longitudinal data collection of infants with congenital uSNHL was included in the PhD project, although there was not enough time to include results in the thesis. The infants with congenital uSNHL in studies III and IV with recordable ABRthrs in the IE (≤60 dB nHL) were invited to a HA fitting shortly after diagnosis, and all children from studies III and IV were part of follow-up measurements of hearing development, speech-language development, and early HA outcomes (exclusion criteria: chromosomal abnormality). Baby illustration by Noun Project; Bianca Teixeira, CC0 1.0, Public Domain Dedication, via Wikimedia Commons, hearing aid picture by Anna Persson.

Further research is also needed into how to best fit HAs to children with uSNHL. Surprisingly, we found that the ABR wave I, III, and V amplitudes as a function of stimulus levels in the group of children with congenital uSNHL in study III were parallel shifted (Figure 16), with significant different interaural ARTs, contrary to what would be expected for cochlear hearing losses with recruitment (Eggermont, 1977; Karlsson et al., 1995). Psychoacoustic measurements of loudness recruitment are difficult to measure in small children. Thus, further research on ABR amplitudes as a function of stimulus levels of other types of SNHL and ARTs, that may be connected to cochlear hearing losses with recruitment are needed in infants as an age-matched comparison. Measurement outcomes of the dynamic range of hearing and recruitment would also be of value in older children with congenital uSNHL to evaluate if the lack of loudness recruitment by neural firing is connected to psychoacoustic measurement outcomes. More research is needed on the connection between HA outcomes, etiology, and affected auditory mechanisms to improve intervention of the children with congenital uSNHL.

HA ventilation effects for children with congenital uSNHL need further study, and the connection to PTTs at low frequencies and aided and unaided SLA. Open ventilation may be a favorable option for the children with congenital uSNHL and efficient low-frequency hearing, to gain access to interaural time difference cues, that are important in horizontal SLA in NH (Wightman & Kistler, 1992).

The effect of directional microphones, adaptive directionality, and noise reduction in children with congenital uSNHL in the outcomes of SLA and listening in noise and competing speech are also subject to future research.

8.3 Causes for congenital uSNHL

Based on the results of studies III and IV we found that broad genetic tests panels are efficient in finding causes for syndromic congenital uSNHL. Exome sequencing, or whole genome sequencing, may be a clinically valuable genetic testing alternative soon to be used clinically. However, several important aspects need careful consideration before exploring the human genome without a targeted approach. When using a broad genetic test panel targeting HL-associated genes, other genetic findings may be discovered. The OtoSCOPE® used in study IV was the first comprehensive genetic test panel for HL that was clinically validated (Shearer et al., 2010; Thorpe & Smith, 2020). OtoSCOPE® was described in 2010 (Shearer et al., 2010), and available in 2012 (Thorpe & Smith, 2020). It is completed by the MORL Clinical Diagnostics Division, a not-for-profit academic laboratory at which genetic experts analyze the results and provide detailed reports with references to relevant research, for help in counseling the patient. We decided to collaborate with MORL in using the OtoSCOPE v.9 based on the knowledge that has been collected over the years in HL with the panel (e.g., Azaiez et al., 2018; Sloan-Heggen et al., 2016), as we together could contribute with more research into the causes for congenital

uSNHL. Another challenge is dual diagnoses in genetic testing, where one genetic diagnosis may mask another genetic diagnosis, or two genetic causes may blend so that it is difficult to determine the cause of HL (Schaefer et al., 2023 February 14). The presence of dual diagnosis in HL highlight the complexity of genetic testing and analysis, and that single gene testing for, e.g., GJB2 non-syndromic SNHL, has its limitations (Schaefer et al., 2023 February 14). Using whole genome sequencing, specific genes or sequences in the genome still need to be targeted for analysis. One large benefit is that you can go back to the results of the whole genome at a later stage, if new research is found. Thus, it will probably be the most common method for testing in the future, although it needs careful ethical considerations for clinical use.

The unknown causes for congenital uSNHL need further study. With MRI and genetic testing the diagnostic yield was 71% (10/14 subjects), indicating that the cause for congenital uSNHL was uncovered in most subjects. Nonetheless, MRI reveals site of lesion, which is usually not the fundamental or basic cause for the HL. For example, the fundamental genetic cause for 1 out of 7 infants with congenital uSNHL with aplasia or severe hypoplasia was found (Townes-Brocks syndrome, Table 4). The remaining 6 out of 7 causes underlying an absent cochlear nerve remain to be revealed. Other factors that may have caused congenital uSNHL are birth complications, as for subject 1 in study III who was diagnosed with brain injury after birth asphyxia, as a probable cause for uSNHL. Nonetheless, this subject was not tested with MRI or genetic testing and other factors could not be ruled out.

The alleged non-syndromic causes for congenital uSNHL remains to be found. One out of 14 children (7%) with alleged non-syndromic congenital uSNHL revealed a genetic cause, and the remaining still have an unknown fundamental cause for the congenital uSNHL. The mother's exposure to ototoxic drugs or other intake during pregnancy was not evaluated in this study. Some drugs are known to cause HL and others remain unknown. I am not aware of any specific exposure during pregnancy in developed countries to be related to congenital uSNHL, except cCMV infection, and rare cases of toxoplasmosis are also associated with SNHL in general (Brown et al., 2009).

We expected to find more cCMV infection causes for congenital uSNHL, even if previous research indicate that cCMV infection-caused SNHL may have an onset after the neonatal period in the majority of subjects (Fowler et al., 1999). Our results stress the important question of how to find the children with uSNHL caused by cCMV infection as early as possible, as they may benefit from a CI in the IE as soon as possible (Benchetrit et al., 2021; Polonenko et al., 2017a; Polonenko et al., 2017b), due to the risks of progression in both ears (Fowler et al., 1997; Fowler et al., 2017; Vos et al., 2021). Our results also stress the importance of finding bilateral EVAs early, as the uSNHL in subject 7 rapidly declined to a bilateral SNHL, with an implanted CI shortly after MRI at 7 months of age, and the need for

a second CI before 2.5 years of age. Our results demonstrate that infants with congenital uSNHL may be born with bilateral EVAs, motivating the use of imaging in the population.

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10 References

AAA. (2013). American Academy of Audiology Clinical Practice Guidelines: Pediatric Amplification. In A. A. o. Audiology (Ed.), (pp. 5-60). Reston (VA): American Academy of Audiology.

Abdala, C., & Kalluri, R. (2017). Towards a joint reflection-distortion otoacoustic emission profile: Results in normal and impaired ears. *The Journal of the Acoustical Society of America*, *142*(2), 812-812.

Aidan, D., Lestang, P., Avan, P., & Bonfils, P. (1997). Characteristics of transient-evoked otoacoustic emissions (TEOES) in neonates. *Acta oto-laryngologica*, *117*(1), 25-30.

Altoè, A., Dewey, J. B., Charaziak, K. K., Oghalai, J. S., & Shera, C. A. (2022). Overturning the mechanisms of cochlear amplification via area deformations of the organ of Corti. *Journal of the Acoustical Society of America*, *152*(4), 2227.

Anne, S., Lieu, J. E. C., & Cohen, M. S. (2017). Speech and Language Consequences of Unilateral Hearing Loss: A Systematic Review. *Otolaryngology--head and neck surgery : official journal of American Academy of Otolaryngology-Head and Neck Surgery*, *157*(4), 572-579.

ANSIS3.5. (1997). ANSIS3.5, 1997. In. New York, USA: American National Standards Institute.

Appachi, S., Specht, J. L., Raol, N., Lieu, J. E. C., Cohen, M. S., Dedhia, K., & Anne, S. (2017). Auditory Outcomes with Hearing Rehabilitation in Children with Unilateral Hearing Loss: A Systematic Review. *Otolaryngology–Head and Neck Surgery*, *157*(4), 565-571.

Arndt, S., Prosse, S., Laszig, R., Wesarg, T., Aschendorff, A., & Hassepass, F. (2015). Cochlear Implantation in Children with Single-Sided Deafness: Does Aetiology and Duration of Deafness Matter? *Audiology and Neurotology*, *20 (1)*(Suppl. 1), 21-30.

Arras, T., Boudewyns, A., Swinnen, F., Zarowski, A., Philips, B., Desloovere, C., . . . van Wieringen, A. (2022). Longitudinal auditory data of children with prelingual single-sided deafness managed with early cochlear implantation. *Scientific Reports*, *12*(1), 9376.

Asp, F., Eskilsson, G., & Berninger, E. (2011). Horizontal sound localization in children with bilateral cochlear implants: effects of auditory experience and age at implantation. *Otology & Neurotology*, *32*(4), 558-564.

Asp, F., Jakobsson, A. M., & Berninger, E. (2018). The effect of simulated unilateral hearing loss on horizontal sound localization accuracy and recognition of speech in spatially separate competing speech. *Hearing Research*, *357*, 54-63.

Asp, F., Olofsson, A., & Berninger, E. (2016). Corneal-Reflection Eye-Tracking Technique for the Assessment of Horizontal Sound Localization Accuracy from 6 Months of Age. *Ear and Hearing*, *37*(2), 104-118.

Azaiez, H., Booth, K. T., Ephraim, S. S., Crone, B., Black-Ziegelbein, E. A., Marini, R. J., . . . Smith, R. J. H. (2018). Genomic Landscape and Mutational Signatures of Deafness-Associated Genes. *American journal of human genetics*, *103*(4), 484-497.

Bagatto, M., Moodie, S., Brown, C., Malandrino, A., Richert, F., Clench, D., & Scollie, S. (2016). Prescribing and Verifying Hearing Aids Applying the American Academy of Audiology Pediatric Amplification Guideline: Protocols and Outcomes from the Ontario Infant Hearing Program. *Journal of the American Academy of Audiology*, *27*(3), 188-203.

Bagatto, M. P., Moodie, S., Scollie, S., Seewald, R., Moodie, S., Pumford, J., & Liu, K. P. (2005). Clinical protocols for hearing instrument fitting in the Desired Sensation Level method. *Trends in Amplification*, *9*(4), 199-226.

Bagatto, M. P., & Scollie, S. D. (2013). Validation of the Parents' Evaluation of Aural/Oral Performance of Children (PEACH) Rating Scale. *Journal of the American Academy of Audiology*, *24*(2), 121-125.

Barbi, M., Binda, S., Caroppo, S., Ambrosetti, U., Corbetta, C., & Sergi, P. (2003). A wider role for congenital cytomegalovirus infection in sensorineural hearing loss. *The pediatric infectious disease journal*, *22*(1), 39-42.

Beck, R. L., Aschendorff, A., Hassepass, F., Wesarg, T., Kroger, S., Jakob, T. F., & Arndt, S. (2017). Cochlear Implantation in Children With Congenital Unilateral Deafness: A Case Series. *Otology & Neurotology*, *38*(10), E570-E576.

Benchetrit, L., Ronner, E. A., Anne, S., & Cohen, M. S. (2021). Cochlear Implantation in Children With Single-Sided Deafness: A Systematic Review and Meta-analysis. *JAMA otolaryngology-- head & neck surgery*, *147*(1), 58-69.

Benchetrit, L., Stenerson, M., Ronner, E. A., Leonard, H. J., Aungst, H., Stiles, D. J., . . . Cohen, M. S. (2022). Hearing Aid Use in Children With Unilateral Hearing Loss: A Randomized Crossover Clinical Trial. *Laryngoscope*, *132*(4), 881-888.

Berninger, E. (2007). Characteristics of normal newborn transient-evoked otoacoustic emissions: ear asymmetries and sex effects. *International Journal of Audiology*, *46*(11), 661-669.

Berninger, E. (2014). Letter to the Editor regarding "Otoacoustic emissions in newborn hearing screening: a systematic review of the effects of different protocols on test outcomes". *International Journal of Pediatric Otorhinolaryngology*, *78*(11), 2022.

Berninger, E., Drott, M., Romanitan, M., Tranebjærg, L., & Hellström, S. (2022). Congenital Nonprofound Bilateral Sensorineural Hearing Loss in Children: Comprehensive Characterization of Auditory Function and Hearing Aid Benefit. *Audiology research*, *12*(5), 539-563.

Berninger, E., & Karlsson, K. K. (1999). Clinical study of Widex Senso on first-time hearing aid users. *Scandinavian Audiology*, *28*(2), 117-125.

Berninger, E., Karlsson, K. K., & Alvan, G. (1998). Quinine reduces the dynamic range of the human auditory system. *Acta oto-laryngologica*, *118*(1), 46-51.

Berninger, E., Olofsson, A., & Leijon, A. (2014). Analysis of click-evoked auditory brainstem responses using time domain cross-correlations between interleaved responses. *Ear and Hearing*, *35*(3), 318-329.

Berninger, E., & Westling, B. (2011). Outcome of a universal newborn hearing-screening programme based on multiple transient-evoked otoacoustic emissions and clinical brainstem response audiometry. *Acta oto-laryngologica*, *131*(7), 728-739.

Bess, F. H., Davis, H., Camarata, S., & Hornsby, B. W. Y. (2020). Listening-Related Fatigue in Children With Unilateral Hearing Loss. *Language, speech, and hearing services in schools*, *51*(1), 84-97.

Bess, F. H., & Tharpe, A. M. (1984). Unilateral hearing impairment in children. *Pediatrics*, *74*(2), 206-216.

Bess, F. H., & Tharpe, A. M. (1986). Case history data on unilaterally hearing-impaired children. *Ear and Hearing*, *7*(1), 14-19.

Bess, F. H., Tharpe, A. M., & Gibler, A. M. (1986). Auditory performance of children with unilateral sensorineural hearing loss. *Ear and Hearing*, *7*(1), 20-26.

Boppana, S. B., Ross, S. A., Novak, Z., Shimamura, M., Tolan, R. W., Jr., Palmer, A. L., . . . Fowler, K. B. (2010). Dried blood spot real-time polymerase chain reaction assays to screen newborns for congenital cytomegalovirus infection. *Jama*, *303*(14), 1375-1382.

Borg, E., Edquist, G., Reinholdson, A. C., Risberg, A., & McAllister, B. (2007). Speech and language development in a population of Swedish hearing-impaired pre-school children, a cross-sectional study. *International Journal of Pediatric Otorhinolaryngology*, *71*(7), 1061-1077.

Borg, E., Risberg, A., McAllister, B., Undemar, B.-M., Edquist, G., Reinholdson, A.-C., . . . Willstedt-Svensson, U. (2002). Language development in hearing-impaired children: Establishment of a reference material for a 'Language test for hearing-impaired children', LATHIC. *International Journal of Pediatric Otorhinolaryngology*, *65*(1), 15-26.

Bovo, R., Martini, A., Agnoletto, M., Beghi, A., Carmignoto, D., Milani, M., & Zangaglia, A. M. (1988). Auditory and academic performance of children with unilateral hearing loss. *Scandinavian Audiology, Supplement*, *30*, 71-74.

Bray, P., & Kemp, D. (1987). An advanced cochlear echo technique suitable for infant screening. *British journal of audiology*, *21*(3), 191-204.

Bredberg, G. (1968). Cellular pattern and nerve supply of the human organ of Corti. *Acta oto-laryngologica*, Suppl 236:231+.

Briggs, L., Davidson, L., & Lieu, J. E. (2011). Outcomes of conventional amplification for pediatric unilateral hearing loss. *Annals of Otology, Rhinology & Laryngology*, *120*(7), 448-454.

Brown, E. D., Chau, J. K., Atashband, S., Westerberg, B. D., & Kozak, F. K. (2009). A systematic review of neonatal toxoplasmosis exposure and sensorineural hearing loss. *International Journal of Pediatric Otorhinolaryngology*, *73*(5), 707-711.

Brownell, W. (1983). Observation on a motile response in isolated outer hair cells. Mechanisms of Hearing. In: Monash University Press, Clayton, Australia.

Brännström, K. J., Ludvigsson, J., Morris, D., & Ibertsson, T. (2014). Clinical note: Validation of the Swedish version of the Parents' Evaluation of Aural/Oral Performance of

Children (PEACH) Rating Scale for normal hearing infants and children. *Hearing, Balance and Communication*, *12*(2), 88-93.

Burke, S. M., van Heesewijk, J. O., Menks, W. M., Klink, D. T., Kreukels, B. P. C., Cohen-Kettenis, P. T., & Bakker, J. (2020). Postnatal Effects of Sex Hormones on Click-Evoked Otoacoustic Emissions: A Study of Adolescents with Gender Dysphoria. *Archives of Sexual Behavior*, *49*(2), 455-465.

Bussé, A. M. L., Hoeve, H. L. J., Nasserinejad, K., Mackey, A. R., Simonsz, H. J., & Goedegebure, A. (2020). Prevalence of permanent neonatal hearing impairment: systematic review and Bayesian meta-analysis. *International Journal of Audiology*, *59*(6), 475-485.

Bussé, A. M. L., Mackey, A. R., Hoeve, H. L. J., Goedegebure, A., Carr, G., Uhlén, I. M., & Simonsz, H. J. (2021). Assessment of hearing screening programmes across 47 countries or regions I: provision of newborn hearing screening. *International Journal of Audiology*, 1-10.

Cama, E., Melchionda, S., Palladino, T., Carella, M., Santarelli, R., Genovese, E., . . . Arslan, E. (2009). Hearing loss features in GJB2 biallelic mutations and GJB2/GJB6 digenic inheritance in a large Italian cohort. *International Journal of Audiology*, *48*(1), 12-17.

Cassidy, J. W., & Ditty, K. M. (2001). Gender differences among newborns on a transient otoacoustic emissions test for hearing. *Journal of Music Therapy*, *38*(1), 28-35.

Chase, S. M., & Young, E. D. (2005). Limited segregation of different types of sound localization information among classes of units in the inferior colliculus. *Journal of Neuroscience*, *25*(33), 7575-7585.

Cheatham, M. A., Goodyear, R. J., Homma, K., Legan, P. K., Korchagina, J., Naskar, S., . . . Richardson, G. P. (2014). Loss of the tectorial membrane protein CEACAM16 enhances spontaneous, stimulus-frequency, and transiently evoked otoacoustic emissions. *Journal of Neuroscience*, *34*(31), 10325-10338.

Ching, T. Y., & Hill, M. (2007). The Parents' Evaluation of Aural/Oral Performance of Children (PEACH) scale: normative data. *Journal of the American Academy of Audiology*, *18*(3), 220-235.

Ching, T. Y. C., Dillon, H., Leigh, G., & Cupples, L. (2018). Learning from the Longitudinal Outcomes of Children with Hearing Impairment (LOCHI) study: summary of 5-year findings and implications. *International Journal of Audiology*, *57*(sup2), S105-S111.

Clemens, L. G., Gladue, B. A., & Coniglio, L. P. (1978). Prenatal endogenous androgenic influences on masculine sexual behavior and genital morphology in male and female rats. *Horm Behav*, *10*(1), 40-53.

Clemmens, C. S., Guidi, J., Caroff, A., Cohn, S. J., Brant, J. A., Laury, A. M., . . . Germiller, J. A. (2013). Unilateral cochlear nerve deficiency in children. *Otolaryngology--head and neck surgery : official journal of American Academy of Otolaryngology-Head and Neck Surgery*, *149*(2), 318-325.

Clopton, B. M., & Silverman, M. S. (1977). Plasticity of binaural interaction. II. Critical period and changes in midline response. *Journal of Neurophysiology*, *40*(6), 1275-1280.

Cohen, J. (1992). A power primer. *Psychological bulletin*, *112*(1), 155-159.

Colvin, I. B., Beale, T., & Harrop-Griffiths, K. (2006). Long-term follow-up of hearing loss in children and young adults with enlarged vestibular aqueducts: relationship to radiologic findings and Pendred syndrome diagnosis. *Laryngoscope*, *116*(11), 2027-2036.

Cox, R. M., & Alexander, G. C. (1995). The abbreviated profile of hearing aid benefit. *Ear and Hearing*, *16*(2), 176-186.

Dallos, P., & Fakler, B. (2002). Prestin, a new type of motor protein. *Nature Reviews Molecular Cell Biology*, *3*(2), 104-111.

Davis, H. (1983). An active process in cochlear mechanics. *Hearing Research*, *9*(1), 79-90.

Dawes, P., Hopkins, R., & Munro, K. J. (2013). Placebo effects in hearing-aid trials are reliable. *International Journal of Audiology*, *52*(7), 472-477.

Dawes, P., Powell, S., & Munro, K. J. (2011). The Placebo Effect and the Influence of Participant Expectation on Hearing Aid Trials. *Ear and Hearing*, *32*(6), 767-774.

Deep, N. L., Gordon, S. A., Shapiro, W. H., Waltzman, S. B., Roland, J. T., & Friedmann, D. R. (2020). Cochlear Implantation in Children with Single-Sided Deafness. *Laryngoscope*, 7.

Dillon, H. (2012). *Hearing aids* (2nd ed.). Boomerang Press : New York : Thieme.

Ead, B., Hale, S., DeAlwis, D., & Lieu, J. E. C. (2013). Pilot study of cognition in children with unilateral hearing loss. *International Journal of Pediatric Otorhinolaryngology*, *77*(11), 1856-1860.

Eggermont, J. J. (1977). Electrocochleography and Recruitment. *Annals of Otology, Rhinology & Laryngology*, *86*(2), 138-149.

Eggermont, J. J., & Don, M. (1986). Mechanisms of central conduction time prolongation in brain-stem auditory evoked potentials. *Archives of neurology*, *43*(2), 116-120.

Ehrmann-Mueller, D., Kurz, A., Kuehn, H., Rak, K., Mlynski, R., Hagen, R., & Shehata-Dieler, W. (2020). Usefulness of cochlear implantation in children with single sided deafness. *International Journal of Pediatric Otorhinolaryngology*, *130*, 6, Article 109808.

Erlandsson, B., Hakanson, H., Ivarsson, A., & Nilsson, P. (1979). Comparison of the hearing threshold measured by manual pure-tone and by self-recording (Békésy) audiometry. *Audiology*, *18*(5), 414-429.

Falconer, D. S., & Mackay, T. F. C. (1996). *Introduction to quantitative genetics*. Longman.

Fellman, J., & Eriksson, A. W. (2006). Weinberg's differential rule reconsidered. *Human Biology*, *78*(3), 253-275.

Ferguson, M. A., Smith, P. A., Davis, A. C., & Lutman, M. E. (2000). Transient-evoked otoacoustic emissions in a representative population sample aged 18 to 25 years. *Audiology*, *39*(3), 125-134.

Fernandez, C., Chasqueira, M.-J., Marques, A., Rodrigues, L., Marçal, M., Tuna, M., . . . Paixão, P. (2022). Lower prevalence of congenital cytomegalovirus infection in Portugal: possible impact of COVID-19 lockdown? *European journal of pediatrics*, *181*(3), 1259-1262.

Fettiplace, R. (2017). Hair Cell Transduction, Tuning, and Synaptic Transmission in the Mammalian Cochlea. *Comprehensive Physiology*, *7*(4), 1197-1227.

Fitzpatrick, E. M., Gaboury, I., Durieux-Smith, A., Coyle, D., Whittingham, J., & Nassrallah, F. (2019). Auditory and language outcomes in children with unilateral hearing loss. *Hearing Research*, *372*, 42-51.

Fowler, K. B., Dahle, A. J., Boppana, S. B., & Pass, R. F. (1999). Newborn hearing screening: Will children with hearing loss caused by congenital cytomegalovirus infection be missed? *The Journal of Pediatrics*, *135*(1), 60-64.

Fowler, K. B., McCollister, F. P., Dahle, A. J., Boppana, S., Britt, W. J., & Pass, R. F. (1997). Progressive and fluctuating sensorineural hearing loss in children with asymptomatic congenital cytomegalovirus infection. *The Journal of Pediatrics*, *130*(4), 624-630.

Fowler, K. B., McCollister, F. P., Sabo, D. L., Shoup, A. G., Owen, K. E., Woodruff, J. L., . . . Boppana, S. B. (2017). A Targeted Approach for Congenital Cytomegalovirus Screening Within Newborn Hearing Screening. *Pediatrics*, *139*(2).

Franklin, D. J., McCoy, M. J., Martin, G. K., & Lonsbury-Martin, B. L. (1992). Test/retest reliability of distortion-product and transiently evoked otoacoustic emissions. *Ear and Hearing*, *13*(6), 417-429.

Ganek, H. V., Cushing, S. L., Papsin, B. C., & Gordon, K. A. (2020). Cochlear Implant Use Remains Consistent Over Time in Children With Single-Sided Deafness. *Ear and Hearing*, *41*(3), 678-685.

García-Santiago, F. A., Martínez-Glez, V., Santos, F., García-Miñaur, S., Mansilla, E., Meneses, A. G., . . . Lapunzina, P. (2015). Analysis of invdupdel(8p) rearrangement: Clinical, cytogenetic and molecular characterization. *American Journal of Medical Genetics Part A*, *167*(5), 1018-1025.

Gardner, M. B., & Gardner, R. S. (1973). Problem of localization in the median plane: effect of pinnae cavity occlusion. *Journal of the Acoustical Society of America*, *53*(2), 400-408.

Gelfand, S. A. (2009). *Essentials of Audiology* (3. ed. ed.). Thieme.

Gelfand, S. A. (2016). *Essentials of Audiology* (4th ed. ed.). Thieme.

Giolas, T. G., & Wark, D. J. (1967). Communication problems associated with unilateral hearing loss. *The Journal of speech and hearing disorders*, *32*(4), 336-343.

Gold, T. (1948). Hearing. II. The Physical Basis of the Action of the Cochlea. *Proceedings of the Royal Society of London. Series B - Biological Sciences*, *135*(881), 492-498.

Gordon, K. A., Henkin, Y., & Kral, A. (2015). Asymmetric Hearing During Development: The Aural Preference Syndrome and Treatment Options. *Pediatrics*, *136*(1), 141-153.

Gordon, K. A., Wong, D. D., & Papsin, B. C. (2013). Bilateral input protects the cortex from unilaterally-driven reorganization in children who are deaf. *Brain*, *136*(Pt 5), 1609-1625.

Gorodensky, J. H., Alemu, R. Z., Gill, S. S., Sandor, M. T., Papsin, B. C., Cushing, S. L., & Gordon, K. A. (2019). Binaural hearing is impaired in children with hearing loss who

use bilateral hearing aids. *Journal of the Acoustical Society of America*, *146*(6), 4352.

Greinwald, J., deAlarcon, A., Meinzen-Derr, J., Greinwald, J., deAlarcon, A., Cohen, A., . . . Meinzen-Derr, J. (2013). Significance of unilateral enlarged vestibular aqueduct. *The Laryngoscope*, *123*(6), 1537-1546.

Gruber, M., Brown, C., Mahadevan, M., & Neeff, M. (2017). Concomitant imaging and genetic findings in children with unilateral sensorineural hearing loss. *The Journal of laryngology and otology*, *131*(8), 688-695.

Guinan, J. J. (2022). Cochlear amplification in the short-wave region by outer hair cells changing organ-of-Corti area to amplify the fluid traveling wave. *Hearing Research*, *426*, 108641.

Guinan, J. J., Jr. (2018). Olivocochlear efferents: Their action, effects, measurement and uses, and the impact of the new conception of cochlear mechanical responses. *Hearing Research*, *362*, 38-47.

Haffey, T., Fowler, N., & Anne, S. (2013). Evaluation of unilateral sensorineural hearing loss in the pediatric patient. *International Journal of Pediatric Otorhinolaryngology*, *77*(6), 955-958.

Hagerman, B. (1982). Sentences for testing speech intelligibility in noise. *Scandinavian Audiology*, *11*(2), 79-87.

Hagerman, B., & Kinnefors, C. (1995). Efficient adaptive methods for measuring speech reception threshold in quiet and in noise. *Scandinavian Audiology*, *24*(1), 71-77.

Hall, J. G. (2003). Twinning. *The Lancet*, *362*(9385), 735-743.

Harris, F. P., Probst, R., & Wenger, R. (1991). Repeatability of transiently evoked otoacoustic emissions in normally hearing humans. *Audiology*, *30*(3), 135-141.

Hartvig Jensen, J., Borre, S., & Johansen, P. A. (1989). Unilateral sensorineural hearing loss in children: cognitive abilities with respect to right/left ear differences. *British journal of audiology*, *23*(3), 215-220.

Hassepass, F., Aschendorff, A., Wesarg, T., Kroger, S., Laszig, R., Beck, R. L., . . . Arndt, S. (2013). Unilateral Deafness in Children: Audiologic and Subjective Assessment of Hearing Ability After Cochlear Implantation. *Otology & Neurotology*, *34*(1), 53-60.

He, W., Kemp, D., & Ren, T. (2018). Timing of the reticular lamina and basilar membrane vibration in living gerbil cochleae. *eLife*, *7*.

Hines, M., Constantinescu, M., & Spencer, D. (2015). Early androgen exposure and human gender development. *Biology of sex differences*, *6*, 3.

Humes, L. E., Allen, S. K., & Bess, F. H. (1980). Horizontal sound localization skills of unilaterally hearing-impaired children. *Audiology*, *19*(6), 508-518.

Huttunen, K., Erixon, E., Löfkvist, U., & Mäki-Torkko, E. (2019). The impact of permanent early-onset unilateral hearing impairment in children – A systematic review. *International Journal of Pediatric Otorhinolaryngology*, *120*, 173-183.

ISO8253-1. (2010). Acoustics — Audiometric test methods — Part 1: Pure-tone air and bone conduction audiometry. In. Geneva, Switzerland: International Organization for Standardization.

Jackler, R. K., & De La Cruz, A. (1989). The large vestibular aqueduct syndrome. *Laryngoscope*, *99*(12), 1238-1242; discussion 1242-1233.

Jerger, J., & Johnson, K. (1988). Interactions of age, gender, and sensorineural hearing loss on ABR latency. *Ear and Hearing*, *9*(4), 168-176.

Jewett, D. L., Romano, M. N., & Williston, J. S. (1970). Human auditory evoked potentials: possible brain stem components detected on the scalp. *Science*, *167*(3924), 1517-1518.

Jiwani, S., Papsin, B. C., & Gordon, K. A. (2016). Early unilateral cochlear implantation promotes mature cortical asymmetries in adolescents who are deaf. *Human brain mapping*, *37*(1), 135-152.

Johansson, M., Asp, F., & Berninger, E. (2020a). Children With Congenital Unilateral Sensorineural Hearing Loss: Effects of Late Hearing Aid Amplification-A Pilot Study. *Ear and Hearing*, *41*(1), 55-66.

Johansson, M., Karltorp, E., Asp, F., & Berninger, E. (2023). A Prospective Study of Genetic Variants in Infants with Congenital Unilateral Sensorineural Hearing Loss. *Journal of Clinical Medicine*, *12*(2), 495.

Johansson, M., Karltorp, E., Edholm, K., Drott, M., & Berninger, E. (2022). A Prospective Study of Etiology and Auditory Profiles in Infants with Congenital Unilateral Sensorineural Hearing Loss. *Journal of Clinical Medicine*, *11*(14), 3966.

Johansson, M., Olofsson, Å., & Berninger, E. (2020b). Twin study of neonatal transient-evoked otoacoustic emissions. *Hearing Research*, *398*, 108108.

Johnsen, N. J., Bagi, P., Parbo, J., & Elberling, C. (1988). Evoked acoustic emissions from the human ear. IV. Final results in 100 neonates. *Scandinavian Audiology*, *17*(1), 27-34.

Johnsen, N. J., & Elberling, C. (1982). Evoked acoustic emissions from the human ear. II. Normative data in young adults and influence of posture. *Scandinavian Audiology*, *11*(2), 69-77.

Johnstone, P. M., Nábělek, A. K., & Robertson, V. S. (2010). Sound localization acuity in children with unilateral hearing loss who wear a hearing aid in the impaired ear. *Journal of the American Academy of Audiology*, *21*(8), 522-534.

Karlsson, K. K., Berninger, E., Gustafsson, L. L., & Alvan, G. (1995). Pronounced quinine-induced cochlear hearing loss. A mechanistic study in one volunteer at multiple stable plasma concentrations. *Journal of Audiological Medicine, 4*, 12-24.

Karltorp, E., Hellström, S., Lewensohn-Fuchs, I., Carlsson-Hansén, E., Carlsson, P. I., & Engman, M. L. (2012). Congenital cytomegalovirus infection – a common cause of hearing loss of unknown aetiology. *Acta Paediatrica*, *101*(8), e357-e362.

Keefe, D. H. (2012). Moments of click-evoked otoacoustic emissions in human ears: group delay and spread, instantaneous frequency and bandwidth. *Journal of the Acoustical Society of America*, *132*(5), 3319-3350.

Keefe, D. H., Gorga, M. P., Jesteadt, W., & Smith, L. M. (2008). Ear asymmetries in middle-ear, cochlear, and brainstem responses in human infants. *Journal of the Acoustical Society of America*, *123*(3), 1504-1512.

Kei, J., McPherson, B., Smyth, V., Latham, S., & Loscher, J. (1997). Transient evoked otoacoustic emissions in infants: effects of gender, ear asymmetry and activity status. *Audiology*, *36*(2), 61-71.

Kemp, D. T. (1978). Stimulated acoustic emissions from within the human auditory system. *Journal of the Acoustical Society of America*, *64*(5), 1386-1391.

Kemp, D. T. (1986). Otoacoustic emissions, travelling waves and cochlear mechanisms. *Hearing Research*, *22*, 95-104.

Kemp, D. T. (2002). Otoacoustic emissions, their origin in cochlear function, and use. *British medical bulletin*, *63*, 223-241.

Kemp, D. T., Ryan, S., & Bray, P. (1990). A guide to the effective use of otoacoustic emissions. *Ear and Hearing*, *11*(2), 93-105.

Khalfa, S., Morlet, T., Micheyl, C., Morgon, A., & Collet, L. (1997). Evidence of peripheral hearing asymmetry in humans: clinical implications. *Acta oto-laryngologica*, *117*(2), 192-196.

Kinsley, C., Miele, J., Konen, C., Ghiraldi, L., & Svare, B. (1986). Intrauterine contiguity influences regulatory activity in adult female and male mice. *Horm Behav*, *20*(1), 7-12.

Kishon-Rabin, L., Kuint, J., Hildesheimer, M., & Ari-Even Roth, D. (2015). Delay in auditory behaviour and preverbal vocalization in infants with unilateral hearing loss. *Developmental Medicine & Child Neurology*, *57*(12), 1129-1136.

Klee, T. M., & Davis-Dansky, E. (1986). A comparison of unilaterally hearing-impaired children and normal-hearing children on a battery of standardized language tests. *Ear and Hearing*, *7*(1), 27-37.

Knudsen, E. I. (2004). Sensitive periods in the development of the brain and behavior. *Journal of Cognitive Neuroscience*, *16*(8), 1412-1425.

Kopun, J. G., & Stelmachowicz, P. G. (1998). Perceived Communication Difficulties of Children With Hearing Loss. *7*(1), 30-38.

Kral, A., Heid, S., Hubka, P., & Tillein, J. (2013a). Unilateral hearing during development: hemispheric specificity in plastic reorganizations. *Frontiers in systems neuroscience*, *7*, 93.

Kral, A., Hubka, P., Heid, S., & Tillein, J. (2013b). Single-sided deafness leads to unilateral aural preference within an early sensitive period. *Brain*, *136*, 180-193.

Kuppler, K., Lewis, M., & Evans, A. K. (2013). A review of unilateral hearing loss and academic performance: Is it time to reassess traditional dogmata? *International Journal of Pediatric Otorhinolaryngology*, *77*(5), 617-622.

Lamminmäki, A., Hines, M., Kuiri-Hänninen, T., Kilpeläinen, L., Dunkel, L., & Sankilampi, U. (2012). Testosterone measured in infancy predicts subsequent sex-typed behavior in boys and in girls. *Hormones and Behavior*, *61*(4), 611-616.

Landersø, R., Nielsen, H. S., & Simonsen, M. (2017). School Starting Age and the Crime-age Profile. *The Economic Journal*, *127*(602), 1096-1118.

Lanzieri, T. M., Chung, W., Flores, M., Blum, P., Caviness, A. C., Bialek, S. R., . . . Demmler-Harrison, G. (2017). Hearing Loss in Children With Asymptomatic Congenital Cytomegalovirus Infection. *Pediatrics*, *139*(3).

Lee, K. H., Larson, D. A., Shott, G., Rasmussen, B., Cohen, A. P., Benton, C., . . . Greinwald, J. H., Jr. (2009). Audiologic and temporal bone imaging findings in patients with sensorineural hearing loss and GJB2 mutations. *Laryngoscope*, *119*(3), 554-558.

Leek, M. R., & Summers, V. (1996). Reduced frequency selectivity and the preservation of spectral contrast in noise. *Journal of the Acoustical Society of America*, *100*(3), 1796-1806.

Liberman, M. C., Gao, J., He, D. Z. Z., Wu, X., Jia, S., & Zuo, J. (2002). Prestin is required for electromotility of the outer hair cell and for the cochlear amplifier. *Nature*, *419*(6904), 300-304.

Lieu, J. E. C. (2004). Speech-Language and Educational Consequences of Unilateral Hearing Loss in Children. *Archives of Otolaryngology–Head & Neck Surgery*, *130*(5), 524-530.

Lieu, J. E. C. (2013). Unilateral hearing loss in children: speech-language and school performance. *B-Ent*, *9*, 107-115.

Lieu, J. E. C., Karzon, R. K., Ead, B., & Tye-Murray, N. (2013). Do Audiologic Characteristics Predict Outcomes in Children With Unilateral Hearing Loss? *Otology & Neurotology*, *34*(9), 1703-1710.

Lieu, J. E. C., Tye-Murray, N., & Fu, Q. (2012). Longitudinal study of children with unilateral hearing loss. *Laryngoscope*, *122*(9), 2088-2095.

Lieu, J. E. C., Tye-Murray, N., Karzon, R. K., & Piccirillo, J. F. (2010). Unilateral Hearing Loss Is Associated With Worse Speech-Language Scores in Children. *Pediatrics*, *125*(6), 1348-1355.

Liming, B. J., Carter, J., Cheng, A., Choo, D., Curotta, J., Carvalho, D., . . . Smith, R. J. (2016). International Pediatric Otolaryngology Group (IPOG) consensus recommendations: Hearing loss in the pediatric patient. *International Journal of Pediatric Otorhinolaryngology*, *90*, 251-258.

Marcoux, A., & Hansen, M. (2003). Ensuring accuracy of the pediatric hearing aid fitting. *Trends in Amplification*, *7*(1), 11-27.

Marshall, L., & Heller, L. M. (1996). Reliability of transient-evoked otoacoustic emissions. *Ear and Hearing*, *17*(3), 237-254.

Martínez-Cruz, C. F., Poblano, A., & Conde-Reyes, M. P. (2009). Cognitive performance of school children with unilateral sensorineural hearing loss. *Archives of medical research*, *40*(5), 374-379.

Martínez-Frías, M. L., Bermejo Sánchez, E., Arroyo Carrera, I., Pérez Fernández, J. L., Pardo Romero, M., Burón Martínez, E., & Hernández Ramón, F. (1999). The Townes-Brocks syndrome in Spain: the epidemiological aspects in a consecutive series of cases. *Anales espanoles de pediatria*, *50*(1), 57-60.

Masuda, S., & Usui, S. (2019). Comparison of the prevalence and features of inner ear malformations in congenital unilateral and bilateral hearing loss. *International Journal of Pediatric Otorhinolaryngology*, *125*, 92-97.

Masuda, S., Usui, S., & Matsunaga, T. (2013). High prevalence of inner-ear and/or internal auditory canal malformations in children with unilateral sensorineural hearing loss. *International Journal of Pediatric Otorhinolaryngology*, *77*(2), 228-232.

McClay, J. E., Booth, T. N., Parry, D. A., Johnson, R., & Roland, P. (2008). Evaluation of pediatric sensorineural hearing loss with magnetic resonance imaging. *Archives of otolaryngology--head & neck surgery*, *134*(9), 945-952.

McFadden, D. (1993a). A masculinizing effect on the auditory systems of human females having male co-twins. *Proceedings of the National Academy of Sciences of the United States of America*, *90*(24), 11900-11904.

McFadden, D. (1993b). A speculation about the parallel ear asymmetries and sex differences in hearing sensitivity and otoacoustic emissions. *Hearing Research*, *68*(2), 143-151.

McFadden, D., & Loehlin, J. C. (1995). On the heritability of spontaneous otoacoustic emissions: A twins study. *Hearing Research*, *85*(1), 181-198.

McFadden, D., Loehlin, J. C., & Pasanen, E. G. (1996). Additional findings on heritability and prenatal masculinization of cochlear mechanisms: click-evoked otoacoustic emissions. *Hearing Research*, *97*(1-2), 102-119.

McKay, S., Gravel, J. S., & Tharpe, A. M. (2008). Amplification considerations for children with minimal or mild bilateral hearing loss and unilateral hearing loss. *Trends in Amplification*, *12*(1), 43-54.

McNamara, H. C., Kane, S. C., Craig, J. M., Short, R. V., & Umstad, M. P. (2016). A review of the mechanisms and evidence for typical and atypical twinning. *American journal of obstetrics and gynecology*, *214*(2), 172-191.

Mehl, A. L., & Thomson, V. (1998). Newborn hearing screening: The great omission. *Pediatrics*, *101*(1), e4, Article e4.

Moore, B. C. (1996). Perceptual consequences of cochlear hearing loss and their implications for the design of hearing aids. *Ear and Hearing*, *17*(2), 133-161.

Moore, B. C. (2008). Basic auditory processes involved in the analysis of speech sounds. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, *363*(1493), 947-963.

Moore, D. R., & Irvine, D. R. (1981). Plasticity of binaural interaction in the cat inferior colliculus. *Brain research*, *208*(1), 198-202.

Moore, D. R., Zobay, O., & Ferguson, M. A. (2019). Minimal and Mild Hearing Loss in Children: Association with Auditory Perception, Cognition, and Communication Problems. *Ear and Hearing*.

Morton, C. C., & Nance, W. E. (2006). Newborn hearing screening--a silent revolution. *The New England journal of medicine*, *354*(20), 2151-2164.

Muzzi, E., Gregori, M., & Orzan, E. (2019). Inner Ear Malformations and Unilateral Sensorineural Hearing Loss-the Elephant in the Room. *Jama Otolaryngology-Head & Neck Surgery*, *145*(9), 874-875.

Møller, A. R., & Jannetta, P. J. (1983). Interpretation of brainstem auditory evoked potentials: results from intracranial recordings in humans. *Scandinavian Audiology*, *12*(2), 125-133.

Nakano, A., Arimoto, Y., & Matsunaga, T. (2013). Cochlear nerve deficiency and associated clinical features in patients with bilateral and unilateral hearing loss. *Otology & Neurotology*, *34*(3), 554-558.

Newton, V. E. (1983). Sound localisation in children with a severe unilateral hearing loss. *Audiology*, *22*(2), 189-198.

Nichols, R. C., & Bilbro, W. C., Jr. (1966). The Diagnosis of Twin Zygosity. *Human Heredity*, *16*(3), 265-275.

Orzan, E., Pizzamiglio, G., Gregori, M., Marchi, R., Torelli, L., & Muzzi, E. (2021). Correlation of cochlear aperture stenosis with cochlear nerve deficiency in congenital unilateral hearing loss and prognostic relevance for cochlear implantation. *Scientific Reports*, *11*(1), 3338.

Orzan, E., Polli, R., Martella, M., Vinanzi, C., Leonardi, M., & Murgia, A. (1999). Molecular genetics applied to clinical practice: the Cx26 hearing impairment. *British journal of audiology*, *33*(5), 291-295.

Paintaud, G., Alvan, G., Berninger, E., Gustafsson, L. L., Idrizbegovic, E., Karlsson, K. K., & Wakelkamp, M. (1994). The concentration-effect relationship of quinine-induced hearing impairment. *Clinical pharmacology and therapeutics*, *55*(3), 317-323.

Parkkonen, L., Fujiki, N., & Mäkelä, J. P. (2009). Sources of auditory brainstem responses revisited: contribution by magnetoencephalography. *Human brain mapping*, *30*(6), 1772-1782.

Paul, A., Marlin, S., Parodi, M., Rouillon, I., Guerlain, J., Pingault, V., . . . Loundon, N. (2017). Unilateral Sensorineural Hearing Loss: Medical Context and Etiology. *Audiology and Neuro-Otology*, *22*(2), 83-88.

Peckham, C. S., & Sheridan, M. D. (1976). Follow-up ?at II years of 46 children with severe unilateral hearing loss at 7 years. *Child: care, health and development*, *2*(2), 107.

Penner, M. J., & Zhang, T. (1997). Prevalence of spontaneous otoacoustic emissions in adults revisited. *Hearing Research*, *103*(1-2), 28-34.

Plontke, S. K., Heider, C., Koesling, S., Hess, S., Bieseke, L., Goetze, G., & Rahne, T. (2013). Cochlear implantation in a child with posttraumatic single-sided deafness. *European Archives of Oto-Rhino-Laryngology*, *270*(5), 1757-1761.

Polley, D. B., Thompson, J. H., & Guo, W. (2013). Brief hearing loss disrupts binaural integration during two early critical periods of auditory cortex development. *Nature Communications*, *4*, 13, Article 2547.

Polonenko, M. J., Gordon, K. A., Cushing, S. L., & Papsin, B. C. (2017a). Cortical organization restored by cochlear implantation in young children with single sided deafness. *Scientific Reports*, *7*(1), 16900.

Polonenko, M. J., Papsin, B. C., & Gordon, K. A. (2017b). Children With Single-Sided Deafness Use Their Cochlear Implant. *Ear and Hearing*, *38*(6), 681-689.

Popelka, G. R., Moore, B. C. J., Fay, R. R., & Popper, A. N. (2016). *Hearing Aids* (Vol. 56). Springer International Publishing AG.

Popescu, M. V., & Polley, D. B. (2010). Monaural Deprivation Disrupts Development of Binaural Selectivity in Auditory Midbrain and Cortex. *Neuron*, *65*(5), 718-731.

Pryor, S. P., Madeo, A. C., Reynolds, J. C., Sarlis, N. J., Arnos, K. S., Nance, W. E., . . . Griffith, A. J. (2005). SLC26A4/PDS genotype-phenotype correlation in hearing loss with enlargement of the vestibular aqueduct (EVA): evidence that Pendred syndrome and non-syndromic EVA are distinct clinical and genetic entities. *Journal of Medical Genetics*, *42*(2), 159.

Purcell, P. L., Cushing, S. L., Papsin, B. C., & Gordon, K. A. (2020). Unilateral Hearing Loss and Single-Sided Deafness in Children: an Update on Diagnosis and Management. *Current Otorhinolaryngology Report*, 1-8.

Purcell, P. L., Shinn, J. R., Coggeshall, S. S., Phillips, G., Paladin, A., Sie, K. C. Y., & Horn, D. L. (2017). Progression of Unilateral Hearing Loss in Children With and Without Ipsilateral Cochlear Nerve Canal Stenosis: A Hazard Analysis. *Otology & Neurotology*, *38*(6).

Purcell, P. L., Shinn, J. R., Davis, G. E., & Sie, K. C. (2016). Children with unilateral hearing loss may have lower intelligence quotient scores: A meta-analysis. *Laryngoscope*, *126*(3), 746-754.

Rance, G., Saunders, K., Carew, P., Johansson, M., & Tan, J. (2014). The use of listening devices to ameliorate auditory deficit in children with autism. *The Journal of Pediatrics*, *164*(2), 352-357.

Rance, G., & Starr, A. (2015). Pathophysiological mechanisms and functional hearing consequences of auditory neuropathy. *Brain*, *138*(Pt 11), 3141-3158.

Ren, T., & He, W. (2020). Two-tone distortion in reticular lamina vibration of the living cochlea. *Communications Biology*, *3*(1), 35.

Ren, T., He, W., & Barr-Gillespie, P. G. (2016). Reverse transduction measured in the living cochlea by low-coherence heterodyne interferometry. *Nature Communications*, *7*, 10282.

Ren, T., He, W., & Kemp, D. (2016). Reticular lamina and basilar membrane vibrations in living mouse cochleae. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(35), 9910.

Rios-Barnes, M., Fontalvo, M. A., Linan, N., Plana, M., Moreno, M., Esteva, C., . . . Alarcón, A. (2022). Letter to the Editor on the original article: Lower prevalence of congenital cytomegalovirus infection in Portugal: possible impact of COVID-19 lockdown? *European journal of pediatrics.*, *181*(3), 1293-1294.

Roeser, R. J., Valente, M., & Hosford-Dunn, H. (2007). *Audiology: Diagnosis*. Thieme.

Rohlfs, A. K., Friedhoff, J., Bohnert, A., Breitfuss, A., Hess, M., Muller, F., . . . Wiesner, T. (2017). Unilateral hearing loss in children: a retrospective study and a review of the current literature. *European journal of pediatrics*, *176*(4), 475-486.

Ropers, F. G., Pham, E. N. B., Kant, S. G., Rotteveel, L. J. C., Rings, E., Verbist, B. M., & Dekkers, O. M. (2019). Assessment of the Clinical Benefit of Imaging in Children With Unilateral Sensorineural Hearing Loss: A Systematic Review and Meta-analysis. *JAMA otolaryngology-- head & neck surgery*, *145*(5), 431-443.

Ruscetta, M. N., Arjmand, E. M., & Pratt, S. R. (2005). Speech recognition abilities in noise for children with severe-to-profound unilateral hearing impairment. *International Journal of Pediatric Otorhinolaryngology*, *69*(6), 771-779.

Sanlaville, D., & Verloes, A. (2007). CHARGE syndrome: an update. *European Journal of Human Genetics*, *15*(4), 389-399.

Schaefer, A., Nishimura, C. J., Frees, K. L., Kolbe, D. L., Booth, K. T., Marini, R. J., . . . Smith, R. J. H. (2023 February 14). *Dual Diagnoses of Genetic Hearing Loss Identified on Multigene Panels: Considerations for Clinical Care and Genetic Counseling* [Poster presentation]. ARO Midwinter meeting 2023, Orlando, Florida, USA.

Schmithorst, V. J., Plante, E., & Holland, S. (2014). Unilateral deafness in children affects development of multi-modal modulation and default mode networks. *Frontiers in Human Neuroscience*, *8*, 164.

Shearer, A. E., DeLuca, A. P., Hildebrand, M. S., Taylor, K. R., Gurrola, J., 2nd, Scherer, S., . . . Smith, R. J. (2010). Comprehensive genetic testing for hereditary hearing loss using massively parallel sequencing. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(49), 21104-21109.

Shera, C. A., & Guinan, J. J., Jr. (1999). Evoked otoacoustic emissions arise by two fundamentally different mechanisms: a taxonomy for mammalian OAEs. *Journal of the Acoustical Society of America*, *105*(2 Pt 1), 782-798.

Silverman, M. S., & Clopton, B. M. (1977). Plasticity of binaural interaction. I. Effect of early auditory deprivation. *Journal of Neurophysiology*, *40*(6), 1266-1274.

Sininger, Y. S., & Abdala, C. (1996). Hearing threshold as measured by auditory brain stem response in human neonates. *Ear and Hearing*, *17*(5), 395-401.

Sininger, Y. S., & Cone-Wesson, B. (2004). Asymmetric cochlear processing mimics hemispheric specialization. *Science*, *305*(5690), 1581.

Sklare, D. A., & Denenberg, L. J. (1987). Interaural attenuation for tubephone insert earphones. *Ear and Hearing*, *8*(5), 298-300.

Sloan-Heggen, C. M., Bierer, A. O., Shearer, A. E., Kolbe, D. L., Nishimura, C. J., Frees, K. L., . . . Smith, R. J. H. (2016). Comprehensive genetic testing in the clinical evaluation of 1119 patients with hearing loss. *Human Genetics*, *135*(4), 441-450.

Smith, R. J., Bale, J. F., Jr., & White, K. R. (2005). Sensorineural hearing loss in children. *The Lancet*, *365*(9462), 879-890.

Smith, R. J. H., Iwasa, Y., & Schaefer, A. M. (2020). Pendred Syndrome / Nonsyndromic Enlarged Vestibular Aqueduct. In *In: Adam MP, Everman DB, Mirzaa GM, et al., editors. GeneReviews® [Internet].* Seattle (WA): University of Washington, Seattle; 1993-2022.

Sohmer, H., & Feinmesser, M. (1967). Cochlear action potentials recorded from the external ear in man. *The Annals of otology, rhinology, and laryngology*, *76*(2), 427-435.

Springer, S., & Searleman, A. (1978). Laterality in twins: The relationship between handedness and hemispheric asymmetry for speech. *Behavior Genetics*, *8*(4), 349-357.

Statistics Sweden. (2022). *Retrieved 2022-10-28 from* https://www.scb.se/en.

Stein, D. M. (1983). Psychosocial characteristics of school-age children with unilateral hearing losses. *Journal of the Academy of Rehabilitative Audiology*(16), 12-22.

Talmadge, C. L., Long, G. R., Murphy, W. J., & Tubis, A. (1993). New off-line method for detecting spontaneous otoacoustic emissions in human subjects. *Hearing Research*, *71*(1-2), 170-182.

Tavora-Vieira, D., & Rajan, G. P. (2015). Cochlear Implantation in Children With Congenital and Noncongenital Unilateral Deafness: A Case Series. *Otology & Neurotology*, *36*(2), 235-239.

Tavora-Vieira, D., & Rajan, G. P. (2016). Cochlear implantation in children with congenital unilateral deafness: Mid-term follow-up outcomes. *Eur Ann Otorhinolaryngol Head Neck Dis*, *133*, 12-14.

Tharpe, A. M. (2008). Unilateral and mild bilateral hearing loss in children: past and current perspectives. *Trends in Amplification*, *12*(1), 7-15.

Thomas, J. P., Neumann, K., Dazert, S., & Voelter, C. (2017). Cochlear Implantation in Children With Congenital Single-Sided Deafness. *Otology & Neurotology*, *38*(4), 496-503.

Thornton, A. R., Marotta, N., & Kennedy, C. R. (2003). The order of testing effect in otoacoustic emissions and its consequences for sex and ear differences in neonates. *Hearing Research*, *184*(1-2), 123-130.

Thorpe, R. K., & Smith, R. J. H. (2020). Future directions for screening and treatment in congenital hearing loss. *Precis Clin Med*, *3*(3), 175-186.

Tillein, J., Hubka, P., & Kral, A. (2016). Monaural Congenital Deafness Affects Aural Dominance and Degrades Binaural Processing. *Cerebral Cortex*, *26*(4), 1762-1777.

Tropitzsch, A., Schade-Mann, T., Gamerdinger, P., Dofek, S., Schulte, B., Schulze, M., . . . Holderried, M. (2022). Diagnostic Yield of Targeted Hearing Loss Gene Panel Sequencing in a Large German Cohort With a Balanced Age Distribution from a Single Diagnostic Center: An Eight-year Study. *Ear and Hearing*, *43*(3), 1049-1066.

Updike, C. D. (1994). Comparison of FM auditory trainers, CROS aids, and personal amplification in unilaterally hearing impaired children. *Journal of the American Academy of Audiology*, *5*(3), 204-209.

Usami, S. I., Kitoh, R., Moteki, H., Nishio, S. Y., Kitano, T., Kobayashi, M., . . . Watanabe, K. (2017). Etiology of single-sided deafness and asymmetrical hearing loss. *Acta oto-laryngologica*, *137*(sup565), S2-s7.

van Beeck Calkoen, E. A., Engel, M. S. D., van de Kamp, J. M., Yntema, H. G., Goverts, S. T., Mulder, M. F., . . . Hensen, E. F. (2019). The etiological evaluation of sensorineural hearing loss in children. *European journal of pediatrics*, *178*(8), 1195-1205.

van Beeck Calkoen, E. A., Sanchez Aliaga, E., Merkus, P., Smit, C. F., van de Kamp, J. M., Mulder, M. F., . . . Hensen, E. F. (2017). High prevalence of abnormalities on CT and

MR imaging in children with unilateral sensorineural hearing loss irrespective of age or degree of hearing loss. *International Journal of Pediatric Otorhinolaryngology*, *97*, 185-191.

Van den Bogaert, T., Klasen, T. J., Moonen, M., Van Deun, L., & Wouters, J. (2006). Horizontal localization with bilateral hearing aids: without is better than with. *Journal of the Acoustical Society of America*, *119*(1), 515-526.

Van Deun, L., van Wieringen, A., Van den Bogaert, T., Scherf, F., Offeciers, F. E., Van de Heyning, P. H., . . . Wouters, J. (2009). Sound localization, sound lateralization, and binaural masking level differences in young children with normal hearing. *Ear and Hearing*, *30*(2), 178-190.

vom Saal, F. S., & Bronson, F. H. (1980). Sexual characteristics of adult female mice are correlated with their blood testosterone levels during prenatal development. *Science*, *208*(4444), 597-599.

von Békésy, G., & Wever, E. G. (1960). *Experiments in Hearing*. McGraw-Hill.

Vos, B., Noll, D., Whittingham, J., Pigeon, M., Bagatto, M., & Fitzpatrick, E. M. (2021). Cytomegalovirus-A Risk Factor for Childhood Hearing Loss: A Systematic Review. *Ear and Hearing*, *42*(6), 1447-1461.

Wang, L., Xu, X., Zhang, H., Qian, J., & Zhu, J. (2015). Dried blood spots PCR assays to screen congenital cytomegalovirus infection: a meta-analysis. *Virology journal*, *12*, 60.

WHO. (2022). *Deafness and hearing loss – Prevalence*. Retrieved 2022-12-05 from https://www.who.int/health-topics/hearing-loss#tab=tab_2.

Wightman, F. L., & Kistler, D. J. (1992). The dominant role of low-frequency interaural time differences in sound localization. *Journal of the Acoustical Society of America*, *91*(3), 1648-1661.

Wright, A. (1984). Dimensions of the cochlear stereocilia in man and the guinea pig. *Hearing Research*, *13*(1), 89-98.

Wright, A., Davis, A., Bredberg, G., Ülehlová, L., & Spencer, H. (1987). Hair Cell Distributions in the Normal Human Cochlea: A Report of a European Working Group. *Acta oto-laryngologica*, *104*(sup436), 15-24.

Zhang, G. Y., Yang, M., Liu, B., Huang, Z. C., Li, J., Chen, J. Y., . . . Teng, G. J. (2016). Changes of the directional brain networks related with brain plasticity in patients with long-term unilateral sensorineural hearing loss. *Neuroscience*, *313*, 149-161.

Zweig, G., & Shera, C. A. (1995). The origin of periodicity in the spectrum of evoked otoacoustic emissions. *Journal of the Acoustical Society of America*, *98*(4), 2018-2047.