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Genetic hearing loss in children

Genetic variation and parental experiences of genetic diagnostics

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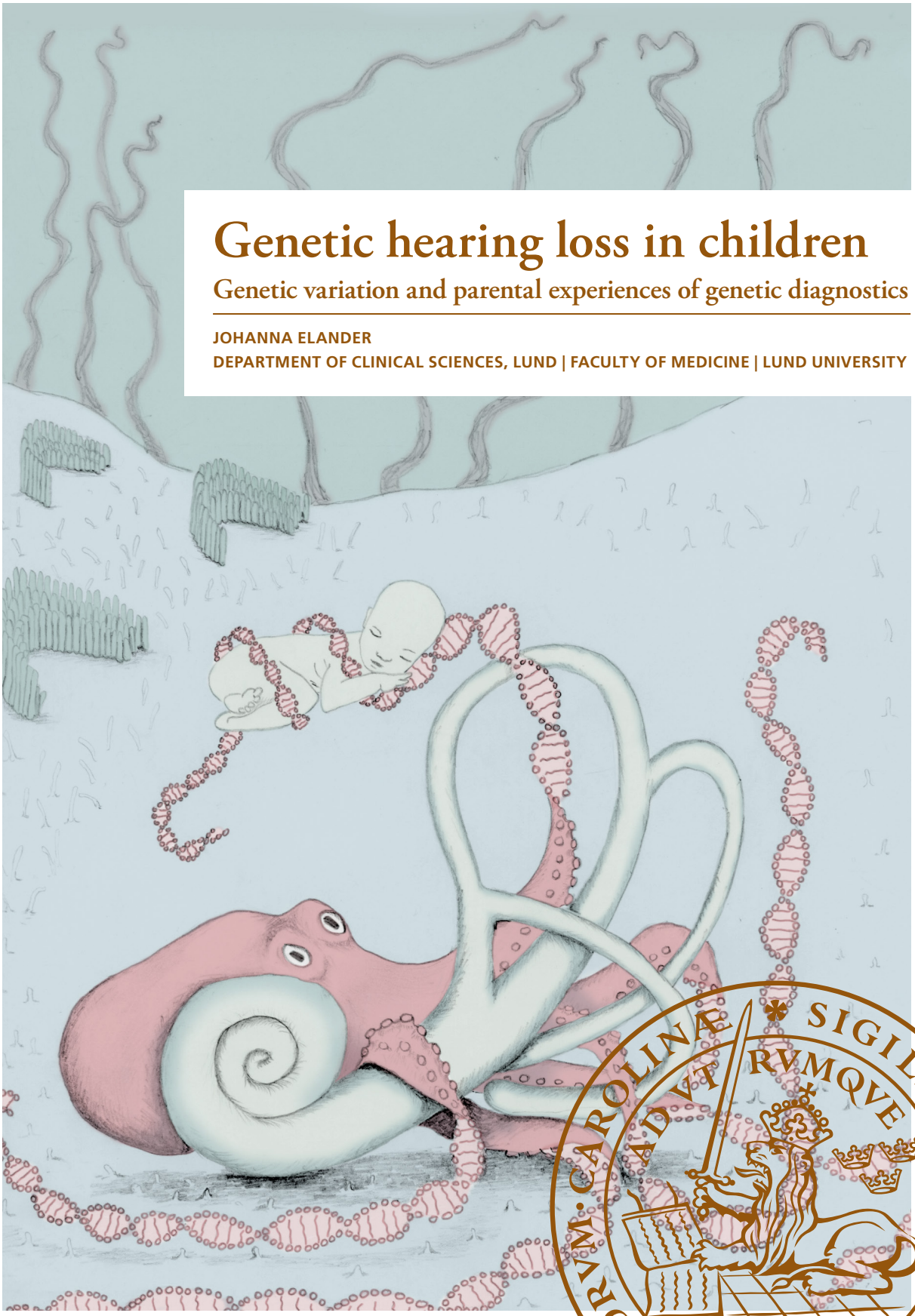


HEARSEQ

Genetic hearing loss in children

Genetic variation and parental experiences of genetic diagnostics

Johanna Elander

LUND
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Abstract:

Introduction: Hearing loss (HL) represents the most prevalent form of sensory impairment, with an incidence of approximately one to two cases per 1,000 newborns. The prevalence increases with age. In the majority of cases, a genetic etiology is present. The genetic background is heterogeneous, with 156 genes associated with non-syndromic HL and hundreds of genes associated with syndromic HL. Additionally, HL can be associated with pathogenic variants (PVs) in mitochondrial DNA. The experiences and evaluations of patients, families, and parents regarding genetic sequencing in relation to hearing loss are sparsely investigated.

Method: The HL characteristics of a large cohort (n=197) of patients with primary mitochondrial disease (PMD) have been retrospectively studied (study I). Prospectively, the genetic variation in patients examined with whole-exome sequencing (WES) (study II) and whole-genome sequencing (WGS) (n=96) (study III) was analyzed. A questionnaire study was conducted to examine parental views on genetic sequencing related to HL as a pilot study (study II). A qualitative interview study, with parents of children with HL examined using WGS, was exploring parental experiences with whole genome sequencing (study IV).

Results: Among patients with PMD, more than a quarter (27%) had HL, primarily with onset in school-age, adolescent, and early adulthood (study I).

The overall genetic yield in patients examined with WES/WGS was 43% (studies II, III). In every second patient (48%) with moderate to profound HL, a genetic cause was identified. The diagnostic yield was slightly higher among prelingual cases (52%). Pathogenic variants were identified in 25 different genes, and an autosomal recessive inheritance pattern dominated. PVs associated with isolated HL were identified in 26 cases and syndromic HL in 15 cases. Almost half of the patients (n=43) had parents from another country of birth, primarily from countries in the Middle East (n=29). The diagnostic yield in this group was 58% (n=17/29), and a homozygous autosomal recessive inheritance pattern dominated 82% (n=14/17).
In the thematic analysis, three global themes were identified, all of which centered on the concept of knowledge (study IV). The first identified global theme was that limited knowledge, both regarding information and uncertainties within the test result, creates uncertainty. In the second global theme, parents acknowledge the importance of knowledge on both a personal and societal level, as well as its practical implications. Parents identified that knowledge adds complexity and that choices related to knowledge can be challenging as the third global theme.

Conclusion: PMD should be considered in cases of postlingual HL. In cases of prelingual moderate to profound HL, a genetic cause could be identified in more than half of the patients, and the genetic background was varied. Additionally, parents found genetic testing to be both personally valuable and practically useful. Thus, considered important for the family and the future.

Keywords: Genetic hearing loss, non-syndromic hearing loss, syndromic hearing loss, whole exome sequencing, whole genome sequencing, parental experiences, thematic analysis

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Abbreviations

HL	Hearing loss
SNHL	Sensorineural hearing loss
GBD	Global Burden of Diseases, Injuries, and Risk Factors
dB	Decibel hearing level
4fPTA	4 Frequencies pure tone average
OAE	Otoacustic emission
ABR	Auditory brainstem response
ASSR	Auditory steady state responses
DNA	Deoxyribonucleic acid
A	Adenine
T	Thymine
C	Cytosine
G	Guanine
mRNA	Messenger ribonucleic acid
RNA	Ribonucleic acid
nDNA	Nuclear deoxyribonucleic acid
mtDNA	Mitochondrial deoxyribonucleic acid
CNV	Copy number variants
ACMG	American College of Medical Genetics and Genomics
MAF	Minor allele frequency
VUS	Variant of uncertain significance
PV	Pathogenic variant
RP	Retinitis pigmentosa
ERG	Electroretinography
RTD	Riboflavin transporter deficiency
PMD	Primary mitochondrial diseases
ATP	Adenosine triphosphate
SLSMD	Single large scale mDNA deletions
MPS	Massive parallel sequencing
PCR	Polymeras chain reaction
CI	Cochear implant
WES	Whole exome sequencing
WGS	Whole genome sequencing
PREM	Patient-related experiences measures

Original articles

I. **Pathogenic mtDNA variants, in particular single large-scale mtDNA deletions, are strongly associated with post-lingual onset sensorineural hearing loss in primary mitochondrial disease**. Elander, J., McCormick, E. M., Varendh, M., Stenfeldt, K., Ganetzky, R. D., Goldstein, A., Zolkipli-Cunningham, Z., MacMullen, L. E., Xiao, R., Falk, M. J., & Ehinger, J. K. (2022). Mol Genet Metab, 137(3), 230-238. https://doi.org/10.1016/j.ymgme.2022.09.002

II. **Extended genetic diagnostics for children with profound sensorineural hearing loss by implementing massive parallel sequencing. Diagnostic outcome, family experience and clinical implementation**. Elander, J., Ullmark, T., Ehrencrona, H., Jonson, T., Piccinelli, P., Samuelsson, S., Lowgren, K., Falkenius-Schmidt, K., Ehinger, J., Stenfeldt, K., & Varendh, M. (2022). Int J Pediatr Otorhinolaryngol, 159, 111218. https://doi.org/10.1016/j.ijporl.2022.111218

III. **Diagnostic Yield and Genetic Variation in 85 Swedish Patients with Mild to Profound Hearing Loss Analyzed by Whole Genome Sequencing.** Elander, J., Ullmark, T., Lowgren, K., Stenfeldt, K., Falkenius-Schmidt, K., Lofgren, M., Castiglione, A., Busi, M., Jonson, T., Ivarsson, S., Ehrencrona, H., Ehinger, J. K., & Varendh, M. (2025). J Otolaryngol Head Neck Surg, 54, 19160216251345471. https://doi.org/10.1177/19160216251345471

IV. **Parental experience of whole genome sequencing for children with sensorineural hearing loss.** Johanna Elander (JE), Maria Värendh, Johannes K Ehinger, Karin Stenfeldt*, Stephen Widén (SW)* (Manuscript submitted) *Contributed equally

Thesis at a glance

	AIM	STUDY DESIGN	RESULTS	CONCLUSION
I	Evaluate the characteristics of hearing loss (HL) in patients with primary mitochondrial disease (PMD), based on genetic background.	**Retrospective cohort study** 193 patients with PMD confirmed at the Children's Hospital of Philadelphia.	27% had HL. All patients with mitochondrial DNA (mtDNA) variants had postlingual hearing loss.	HL is common in PMD. When onset of HL occurs in school-aged children, adolescents, and young adults mtDNA pathology should be considered.
II	Describe the genetic variation in Swedish children with profound sensorineural HL. Evaluate parental experience and describe implementation of whole-exome sequnecing (WES).	**Prospective pilot study** Eleven children with profound HL were genetically tested with WES. HearSeq gene panel used. A patient-related experiences measures (PREM) questionnaire was also used.	Genetic findings of interest was found in 55%. In 45% it was related to Usher syndrome. A confirmed genetic diagnosis was identified in 27%. All participants recommended genetic testing for other families in the same situation.	We showed a high diagnostic yield in patients examined with WES. The parents found the testing valuable.
III	Describe the genetic variation in a Swedish population with mild to profound SNHL. Identify factors relevant for a higher diagnostic yield.	**Prospective cohort study** 85 patients with mild to profound HL examined with whole genome sequencing (WGS), analysed with HearSeq gene panel.	The total genetic diagnostic yield was 45% with pathogenic variants in 24 different genes. Children with prelingual moderate to profound SNHL had a diagnostic yield of 60% (n= 31/52).	A genetic cause was identified in almost half of the patients. Prelingual onset of SNHL favored a higher diagnostic yield. In children, a genetic diagnosis was useful for prognostic purposes.
IV	Explore parental experiences of genetic testing of children with HL.	**Qualitative thematic interview study** 10 parents to children examined with WGS.	Three global themes: 1. Limited knowledge creates uncertainty. 2. Genetic knowledge is considered important for the family and the future. 3. Knowledge adds complexity and can be challenging.	Parents experienced genetic testing as personally valuable and practically useful, even if no treatment options were available. Conversely, ambiguous or unreliable results can cause difficulties.

The genetic background of sensorineural hearing loss

Sensorineural hearing loss and the inner ear

Hearing loss (HL) is the most common sensory impairment in newborns, affecting approximately one to two per thousand newborns, and the prevalence increases with age[1,2]. According to a study from a Swedish population in 2020[2], which included a comparison with several studies from high-income countries, the prevalence of hearing impairment in children has remained the same over the past four decades. This suggests that the cause of hearing impairment in children is mainly genetic. Although there are other potential causes, such as infections, ototoxic drugs, prematurity, and trauma, the majority of children (~70%) with sensorineural hearing loss (SNHL) in high-income countries are estimated to have a genetic cause[1,3,4].

Nevertheless, globally, the prevalence of hearing impairment is increasing, and according to the Global Burden of Diseases, Injuries, and Risk Factors (GBD) Study[5], the HL expert group estimates that 700 million people will require hearing rehabilitation by 2050. They conclude that globally, HL is the third largest cause of years lived with disability after low back pain and migraine. The increased prevalence is related to population growth and aging, while age-standardized prevalence remains stable.

Hearing impairment can be either conductive or sensorineural. Conductive HL is localized to the outer or middle ear, whereas SNHL originates in the cochlea, the cochlear nerve, or central auditory pathways. In this thesis, the focus will be on SNHL.

HL is defined as hearing threshold >20 decibel hearing level (dB) in pure-tone average on audiometric threshold of 0.5, 1, 2, and 4 kHz (4fPTA) and graded from mild to profound. There are different classifications of HL, but an updated definition of HL from the GBD expert group[5,6] is available. However, we have chosen to use the WHO classification from 1991 in our studies, as the newer classification is not validated for children. In our studies, HL is defined as mild (21-40 dB), moderate (41-60 dB), severe (61-80 dB), and profound (>80 dB). Since the development of spoken language is dependent on hearing, another important definition of HL is whether HL begins before or after normal speech development, known as pre- or

postlingual HL. In our studies, we have defined prelingual HL as the onset of HL before the age of 2 years. In the era before prenatal hearing screening, the definition of prelingual HL was the onset of HL before 5 years. This is probably related to the fact that HL was diagnosed later and first became obvious after delayed language development.

The inner ear is a complex system in which sound waves are transmitted in three fluid-filled, spiral-formed compartments, the scala vestibuli, scala media, and scala tympani, of the cochlea (**Figure 1**). The differences in ion concentration in the perilymph of the scala vestibuli and scala tympani and in the endolymph of the scala media allow a positive electrochemical potential to be maintained. Ion channels, including both active and passive, ligand-gated, and voltage-gated channels, regulate the concentration of ions[7]. Stria vascularis is a multilayered epithelium, where most of the essential potassium channels, for maintenance of the potassium level in the endolymph are situated[7,8]. In the scala media, the organ of Corti is located on the basilar membrane, which separates the scala tympani from the scala media, while Reissner´s membrane separates the scala vestibuli from the scala media. In the organ of Corti, layers of inner and outer hair cells are covered by the tectorial membrane. Vibrations from the sound waves are transmitted to the basilar membrane, leading to a consecutive movement between the tectorial membrane and the stereocilia, mechanosensitive ion channels open, hair cells depolarize and trigger nerve impulses from the sensory cells, the inner hair cells[8]. The nerve impulses follow the spiral ganglion in the central part of the cochlea, the modiolus, and continue along the cochlear nerve[9]. Multiple proteins are necessary to form the complex structures of the cochlea and to enable sound to be transmitted to the auditory cortex, all the way along the auditory pathway.

Evaluation of hearing function

There are numerous ways to measure hearing function, with both subjective and objective modalities. The measurements complement each other and must be performed in an age-appropriate manner. Small children are often audibly evaluated with objective measures, such as Otoacoustic Emission (OAE), Auditory Brainstem Response (ABR), and Auditory Steady State Responses (ASSR), whereas older children and adults can participate in subjective methods and perform pure tone audiometry (visual reinforcement or conditioned play audiometry when needed) and speech audiometry.

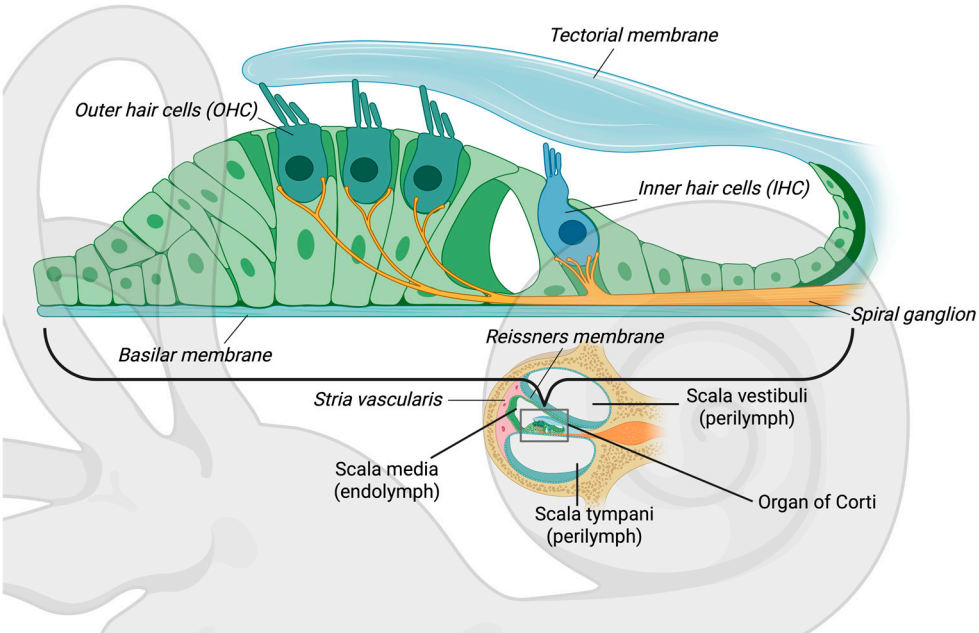

Figure 1. The complex structures of the inner ear with the Organ of Corti
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Genetic hearing loss

The auditory system is complex, and many proteins are necessary for function. Consequently, there are many areas in the deoxyribonucleic acid (DNA) sequence, the blueprint for proteins, where variants can be potentially pathogenic.

Basic Genetics

Understanding hearing genetics requires a basic understanding of genetics, which is crucial for comprehending both the benefits and limitations of various genetic investigations and test results. The genome is found in the nuclei, but the mitochondria also contain DNA.

The DNA molecule is composed of nucleotides in a double-stranded helical structure. Each nucleotide has a base, where the base adenine (A) always forms a base pair with thymine (T) and guanine (G) with cytosine (C). The consistency of pairing is the key to replication during cell division and the transcription process. The DNA code is transcribed by enzymes into a single-stranded messenger

ribonucleic acid (mRNA) molecule, and each triplet of bases of the mRNA is then translated into an amino acid[10]. A sequence of amino acids makes a protein, our building blocks for life. DNA is organized into 23 pairs of chromosomes, with one chromosome in each pair coming from the mother and the other from the father. One of the pairs determines the sex (XX and XY), while the rest are autosomal and numbered by size, with the largest being first[10]. Genes are located on chromosomes; hence, each gene comes in two versions, and each version, or specific location of the DNA sequence, is called an allele.

Although the DNA sequence serves as a blueprint for protein production, only a fraction, the exons, of the DNA is protein-coding, while most of the DNA, including the introns, is non-protein-coding. The function of the introns is not fully understood, even though some parts of the intron code regulate the process of transcription and gene expression[11]. For example, variation at splice sites, the breakpoint between the intron and exon, can affect the transcript of mRNA and thus, gene expression. This is a complex process involving small nuclear RNA and splicing protein factors, which form spliceosomes, transforming pre-mRNA to mature mRNA[12].

The human nuclear genome is vast, comprising approximately three billion base pairs and consisting of around 21.000 genes. There are approximately three million variations in the genome when comparing the nuclear DNA (nDNA) of one person with the reference genome, and most of these variants are considered natural variation between individuals. In fact, only a small fraction of the variants are pathogenic or likely pathogenic[13].

In addition to the nuclear genome, the mitochondria contain mitochondrial DNA (mtDNA). The mtDNA is, in comparison with nDNA, a small DNA strand composed of 16.569 base pairs that harbors 37 genes[14]. The genes code for 13 polypeptides used in the complexes in the respiratory chain, 22 transfer RNAs, as well as two ribosomal RNAs (12S and 16S) essential for mitochondrial translation[14,15].

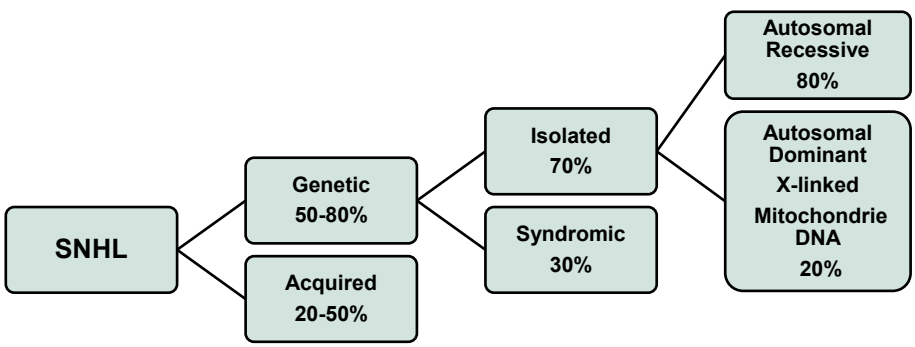
Variants and classification

There are many different types of variants in the genome, which can lead to both changes in DNA structure and protein expression. Some variants, such as deletions in one or more exons, frameshift or nonsense variants, can lead to loss of function, while other variants can affect a single amino acid and, for example, impair the function of the protein. Deletions or duplications, where a part of the DNA is missing or repeated, lead to copy number variants (CNV), whereas translocations and inversions are copy number neutral structural rearrangements. Structural variants can be associated with normal genomic variation depending on their location and gene expression[16]. A reference genome (GRCh38) is used, along with

databases, to identify genetic variation. The variants are graded according to the American College of Medical Genetics and Genomics (ACMG) criteria and assessed based on whether there is evidence of benignity or pathogenicity[17]. Evidence supporting benignity is, for example, a variant that is common in the population, i.e., has a high minor allele frequency (MAF). Whereas a variant that segregates with disease in a family, or is de novo in a family with only one affected family member, supports the pathogenicity of that variant. The variants are classified as benign (1), likely benign (2), variant of uncertain significance (VUS, 3), likely pathogenic (4), or pathogenic (5)[17]. The ACMG classifications are adapted for HL[18] by the HL variants Curation Expert Panel, an international expert group founded by Clinical Genome Resource (ClinGen)[19].

Genes and inheritance

HL is heterogeneous, and according to the hereditary hearing loss homepage[20], 156 non-syndromic HL genes have been identified, and several hundred genes can be involved when HL is related to a syndrome[21,22]. Syndromes where HL is the predominant or the first symptom are sometimes called non-syndromic HL mimics, and these are the syndromes in focus in this thesis. Among children with genetic SNHL, around 70% have isolated[23] SNHL, and in the remaining cases (30%), a syndromic cause can be identified. Autosomal recessive inheritance, where both alleles in a gene have to have pathogenic variants (PVs) to cause disease, is the most common form (~80%). Inheritance pattern can be autosomal dominant, where a PV in one allele causes disease (~19%). Additionally, an X-linked inheritance pattern exists, where the PV is located on the X chromosome and commonly affects males to a greater extent than females (<1%). Maternally inherited conditions (mitochondrial) are due to the fact that the mitochondria are passed on with the oocyte, and thus PVs in mtDNA originate from the mother (<1%)[3,24,25] (**Flowchart 1**).



Flowchart 1. Sensorineural hearing loss and expected etiology

When HL onset occurs later in life or in adulthood, the proportion of autosomal dominant inheritance increases[26]. Autosomal recessive inheritance patterns can be homozygous, with two identical variants on the two alleles of the gene, or compound heterozygous, with two different variants on the two alleles affecting the same gene. In the latter case, with two different variants, the investigation must ensure that the variants are localized on different alleles, so-called 'in trans', to cause disease. If the two variants are 'in cis', on the same allele, the second allele is wild-type and thus functional. Sometimes there is a need for parental testing to confirm the relationship between variants and allele localization.

Isolated or non-syndromic sensorineural hearing loss with nuclear origin

Many different genes can be involved in isolated HL, but variants in *GJB2* are the most common, and the prevalence worldwide among people with HL is between 10 and 15%. Among patients with autosomal recessive non-syndromic HL, the prevalence is higher (20%). Although the variants differ between populations, the *GJB2* variant c.35delG dominates in the European and Middle Eastern population[27,28]. *GJB2* codes for the protein Connexin 26. Six subunits of Connexins form a hexameric structure called a hemichannel, which enables the connection between the intracellular and extracellular spaces. Two docked hemichannels connect two cells and form a gap junction, a large pore essential for the intercellular signalling of both small and large molecules. There are different types of Connexins that affect various tissues in the body. Connexin 26 predominates in all non-sensory cells of the cochlea[29].

In different populations around the world, the distribution of identified genes causing isolated HL cases varies. *STRC* and *MYO7A*[3,30-33] are recurrent in many studies and have been of particular interest in our cohort.

Pathology in *STRC* is often due to a CNV, a deletion, which also covers *CATSPER2*, a gene that codes for a sperm specific ion channel. This gene plays a role in sperm motility and is thus related to infertility in men. In a study from Japan, that examined nearly ten thousand people with HL, they identified *STRC*-associated HL in almost 3% and in more than 75% of the cases, there was a deletion also covering *CATSPER2*[34]. In European cohorts, the prevalence of *STRC* is reported to be higher[35,36].

MYO7A is together with a couple of other genes (*USH1C*, *CDH23*, *PCDH15*, *CIB2,* and *PDZD7*)[37], a gene that is linked to various phenotypes, including isolated SNHL and Usher syndrome (described in the next section). This poses the clinicians in a delicate pedagogic position, with a need for further evaluation with an uncertain outcome, and gives rise to anxiety and stress for the patient and family.

Syndromic hearing loss with nuclear origin

The most common syndromes, mimicking isolated HL, are Pendred and Usher syndrome, accounting for an estimated 4-10%[38,39] and 3-6%[37]of hereditary HL, respectively. As mentioned above, there are numerous syndromes where SNHL is part of the symptoms. There are, for example, syndromes characterized by symptoms affecting the eyes, kidneys, heart, and connective tissue, as well as facial malformations and intellectual disability, depending on the protein that is missing or degraded. The following is a description of a few syndromes, without the aim of being comprehensive, but to provide understanding of the diversity in syndromic SNHL.

Pendred syndrome is an autosomal recessive disorder, where the SNHL is accompanied by inner ear malformations, enlargement of the vestibular aqueduct, Mondini malformation, and goiter[39]. In Pendred syndrome, *SLC26A4* is affected. Variants in the gene are not always related to syndromic HL but may be limited to isolated SNHL and inner ear malformation[40]. There are other genes related to Pendred, for example, *FOXI1* codes for a FOXI1-mediated transcriptional factor, activating *SLC26A4*, and thus, pathogenic variants are related to Pendred syndrome. *SLC26A4* codes for the protein Pendrin, a carrier protein that exchanges chloride for bicarbonate to maintain endolymph homeostasis in the inner ear[41]. Loss of Pendrin results in acidification of the endolymph and inhibition of calcium reabsorption[38].

The second most common syndrome is Usher, a syndrome that, in addition to SNHL, causes progressive visual loss due to retinitis pigmentosa (RP) and vestibular impairment. There are four subtypes, where USH1 is the most severe with profound SNHL and an early onset of visual loss and vestibular dysfunction in childhood. However, USH2 is the most common type with progressive SNHL and visual impairment in adolescence and usually intact vestibular function. USH3 has an onset of progressive SNHL and RP somewhat later in life, and the vestibular dysfunction is affected in every second case[37]. The last type, USH4, is an atypical form with even later onset.

Usher syndrome is associated with variants in at least nine confirmed causative genes[42] and, as mentioned above, several genes may be related to either isolated SNHL or concomitant progressive visual impairment with RP[37]. What the proteins involved have in common is that they affect the stereocilia of the hair cells. They are involved in the connection between stereocilia and the structure of the hair cells as scaffold and junction proteins. These proteins cooperate to ensure normal function and structure of the hair cells, and some proteins are essential as tip links[37,43] (**Figure 2**). Retinal function in suspected RP is examined by electroretinography (ERG), and in young children, this is done under general anesthesia[44-46].

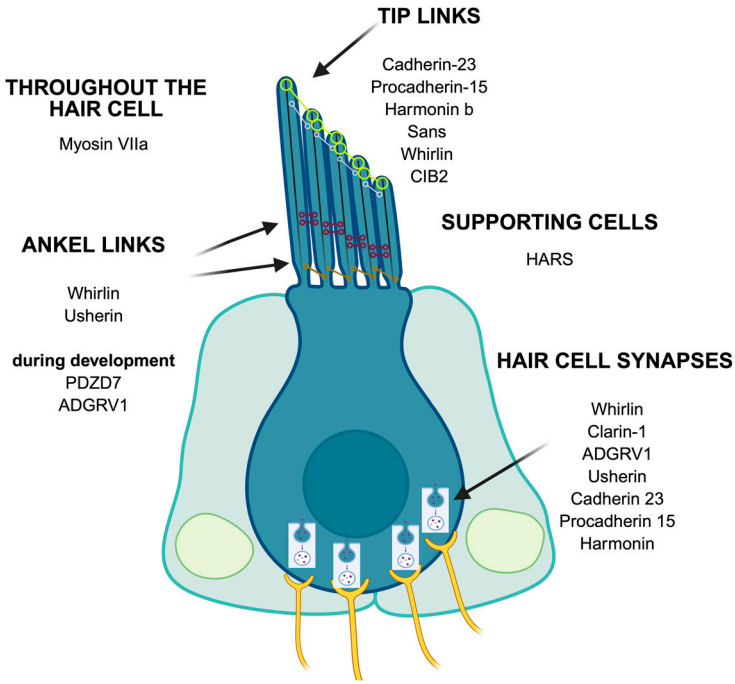


Figure 2. Proteins impaired in to Usher syndrome are crucial for hair cell function and are essential for maintaining the normal structure of the stereocilia. Some proteins are tip links, but also present in other part of the hair cells. Others stabilise the middle and basal parts of the stereocilia or are involved in synaptic activity.
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A collagen disease, causing both a delayed glomerular renal dysfunction and progressive SNHL, is Alport syndrome. It is caused by variants in genes coding for type IV collagens (α3, α4, and α5). The majority have X-linked variants in *COL4A5,* and the remaining cases are associated with autosomal variants in either *COL4A3* or *COL4A4*[47]. These three collagens build a network and have a more restricted tissue distribution than α1 and α2 and are, in addition to the glomerulus, found in the basilar membrane, the stria vascularis, and the spiral ganglia. In the glomerulus, the absence of these collagens results in a focal thinner basement membrane, and in the cochlea, it might affect adhesion of the tectorial membrane and the basilar membrane junction with the spiral ligaments[48].

Branchio-Oto-Renal Syndrome is another syndrome that, in addition to SNHL and branchial cysts and fistulas, also present with structural defects of the outer, middle, and inner ear, as well as renal abnormalities. The inheritance pattern is autosomal dominant, and the *EYA1*[49] and *SIX1*[50] are involved.

Another rare disease, affecting less than 1% of children with SNHL, is Jervell-Lange Nilsen syndrome, where the SNHL is accompanied by a prolonged QT-interval,

ventricular arrhythmias, syncope, and a high risk of sudden death. Potassium channels are affected by variants in *KNCQ1* and *KVNE1,* resulting in difficulties with both endolymphatic homeostasis and ventricular repolarisation[51].

Wardenburg syndrome is an autosomal dominant disease characterized by lateral displacement of the inner canthus (dystopia canthorum), pigmental anomalies (hair, iris, and skin), and SNHL. There are four types, but in types 3 and 4, symptoms from other organs are dominating. Type 2 differs from type 1 in that it does not exhibit dystopia canthorum. *PAX3* and *MITF* are related to type 1 and type 2, respectively[52].

A rare treatable condition is riboflavin transporter deficiency (RTD), which causes progressive peripheral and central neuropathy, with HL being the first symptom in many patients. RTD is associated with PV in *SLC52A2* and *SLC52A3,* genes that encode human riboflavin transporters. Humans are unable to synthesize riboflavin and are therefore dependent on dietary intake and efficient cellular transport. Riboflavin is essential for metabolically active cells, such as nerve cells, and plays a role in mitochondrial oxidative phosphorylation, among other things. High doses of oral riboflavin are an effective treatment for both clinical improvement and disease stabilization[53].

This is a selection of syndromes to increase the understanding of the diversity of syndromes with HNS as one of the symptoms.

Primary mitochondrial disease

HL related to primary mitochondrial disease (PMD) can be either syndromic or non-syndromic.

Tissues and organs with high energy consumption are especially sensitive to mitochondrial dysfunction. Besides muscles and neurons, sensory cells in the retina and in the cochlea are thus vulnerable[54]. Mitochondria are organelles that generate energy as adenosine triphosphate through oxidative phosphorylation within the respiratory chain[25]. Metabolically active cells with a low rate of cell division, as the inner and outer hair cells, as well as cells in stria vascularis, are compromised by a decreased adenosine triphosphate (ATP) production[55,56].

Each cell contains multiple mitochondria, and each mitochondrion has multiple copies of mtDNA[14,15]. If a mtDNA variant affects all mtDNA copies, it is referred to as homoplasmy. When it comes to PVs, there is usually a mixture of wild-type (without PVs) and mutated mtDNA, a phenomenon known as heteroplasmy[25] (**Figure 3**). Heteroplasmy levels vary between different tissues (e.g., muscle, urinary tract epithelial cells, blood), and when the PVs exceed a certain threshold, they cause disease[57].

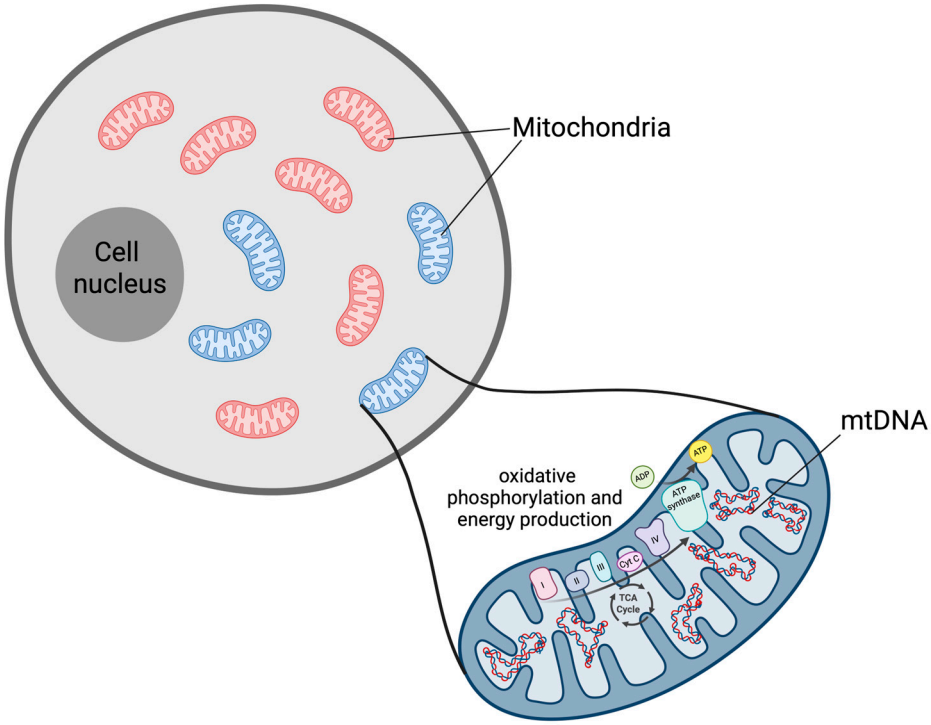


Figure 3. A cell with mitochondria in heteroplasmy and multiple copies of mtDNA in the organelle. Created in BioRender. Elander, J. (2025) https://BioRender.com/zxn3bz8

Although mitochondria have their own mtDNA, most of the proteins essential for mitochondrial structure and function are encoded by nDNA. The mitochondrial oxidative phosphorylation chain consists of five complexes, and the majority of proteins needed are encoded in the nucleus[58,59].

Consequently, PMD can be due to PVs in both the nuclear and the mtDNA. The mtDNA variation can be single large-scale mtDNA deletions (SLSMD) or point mutations. Some nDNA variants lead to mtDNA depletion syndrome, which results in a progressive decline of mtDNA, ultimately resulting in decreased ATP production. Thus, the genetic background in PMD is complex, and PVs in more than 300 genes have been associated with PMD[60]. Dysfunction in the mitochondria can be either uniparentally or biparentally inherited[58]. Paternal inheritance in PMD is due to variants in the nDNA.

Mitochondrial hearing loss

People with PMD often develop bilateral SNHL[56,61,62]. Despite this fact, this group of patients represents a small, almost negligible, part (<1%) of the total group of children with HL due to the rarity of diagnosed PMDs.

HL related to mitochondrial disease can be syndromic or isolated. Syndromic PMDs are often described according to distinct phenotypes (Table 1) and can have different genetic origins. More than one-third of the patients with PMD suffer from HL, and the impairment is described as primarily affecting high frequencies[63].

Table 1. Primary mitochondrial disease, clinical diagnostic groups, and genetic findings

SYNDROME	*TYPICAL FEATURE(S)*	*FREQUENT GENETIC FINDING*
MIDD Maternally inherited diabetes and deafness	Diabetes and hearing loss	m3243A>G
MELAS Mitochondrial encephalomyopathy lactic acidosis and stroke like episodes syndrome	Stroke-like episodes, cardiac involvement, diabetes, hearing loss	m3243A>G
CPEO Chronic progressive external ophthalmoplegia	Ptosis, impaired eye movement	Various nDNA variants, mtDNA point mutations, mtDNA singel large-scale deletions
MERRF Myoclonic encephalopathy with ragged-red fiber	Myoclonus, myopathy, ataxia	m8344A>G
LHON Leber hereditary optic neuropathy	Blindness (optic neuropathy)	Various mtDNA mutations
Leigh syndrome	Severe pediatric encephalopathy	
NARP Neuropathy, Ataxia, Retinitis Pigmentosa	Ataxia, Impaired vision	M8993T>G
DOA Dominant optic atrophy	Blindness (optic neuropathy)	OPA1
KSS Kearns-Sayre syndrome	Ptosis, ophthalmoparesis, ataxia, cardiac conduction defects	Singel large-scale deletions of mtDNA
Others (Alper syndrome, coenzyme Q10 deficiency, MNGIE)		

The most frequent pathological mtDNA variant associated with non-syndromic SNHL is 1555A>G, followed by 1494C>T, which affects the *MT-RNR1* gene, coding for 12SrRNA. Individuals with these variants are sensitive to

aminoglycosides but can also be affected by congenital or later onset of SNHL without exposure to this type of antibiotic. The background to the sensibility is that 12SrRNA resembles the bacterial ribosomes. The altered 12S sRNA sequence in the mitochondrion resembles the 16S rRNA segment of E. coli bacteria, the target site for aminoglycosides[64,65]. As a result, aminoglycoside binds to the mitochondria, disrupting mitochondrial protein synthesis and oxidative phosphorylation, which leads to sensory hair cell damage and HL. Also, variants related to tRNAs are described to be associated with mitochondrial non-syndromic SNHL (7445A>G, 7511T>C, 12201T>C, 7551A>G, 4295A>G, 5783C>T, m.7516delA)[65].

Van Kempen et al. (2022)[61] analysed SNHL in PMD patients according to different clinical diagnosis groups. They concluded that in MIDD, MELAS, NARP, and myopathy patients, SNHL was commonly seen. More detailed descriptions of hearing impairment in PMD, including the time of onset (years), grade (i.e., mild, moderate, severe, profound), and frequency range (Hz), related to genetic etiology, are limited in the literature. To deepen the understanding of HL characteristics in PMD in relation to genetic background, the first part of this thesis was initiated.

Paper 1

A retrospective cohort study of 193 patients with genetically diagnosed primary mitochondrial disease (PMD) seen at the Children's Hospital of Philadelphia Mitochondrial Medicine Frontier Program (June 2008 to September 2019).

Aim

Evaluate the prevalence, severity, and age of onset of HL in patients with PMD, according to the genetic background.

Method

Patients were grouped into categories based on the genetic background of the mitochondrial disease: PVs of mtDNA, single large-scale mitochondrial deletions (SLSMD), and PVs of nDNA (including mtDNA depletion). Hearing was graded from normal (≤20 dB HL) to mild (21-40 dB HL), moderate (41-60 dB HL), and severe/profound (≥61dB HL) on 4fPTA. Age of onset was defined as prelingual (<2 years), preschool age (2-5 years), school age (6-12 years), adolescence (13-19 years), young adult (20-40 years), and middle-aged to elderly (>40 years).

Results

Of the 193 PMD patients, 80 had pathogenic mtDNA gene variants, 24 had SLSMD, and 89 had nDNA variants. Formal audiologic testing was performed in 53% (n=103/193), and in about half of the audibly tested cases (27% n=52/193), HL was confirmed. In SLSMD patients, HL was more common, occurring in 58% (14/24), and there was a significant difference between the groups. In 11 cases, the HL was prelingual, and in all of these cases, the PMD was due to nuclear variants (**Figure 4**).

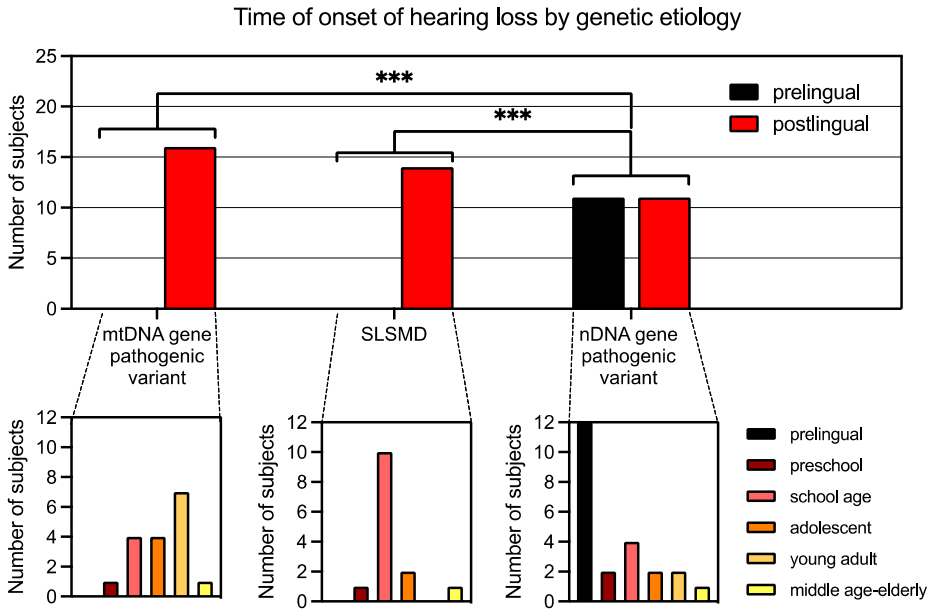


Figure 4. Number of subjects with HL, related to genetic etiology (mtDNA gene pathologic variant, SLSMD, nDNA gene pathologic variant), and time of onset of HL, divided into pre- and postlingual. Additionally, the time of onset of HL according to age group. In all cases with variation in the mtDNA (PVs of mtDNA and SLSMD), the HL was post-lingual. ***Significate difference between the groups, p-value <0.001.

Conclusion

HL was diagnosed in 27% of the 193 patients with PMD, and was more prevalent in SLSMD cases. In all cases with variation in the mtDNA (PVs of mtDNA and SLSMD), the HL was postlingual. In school-aged children, adolescents, and young adults with onset of HL, the mtDNA etiology should therefore be considered, especially if it occurs in combination with other symptoms.

Methods of DNA sequencing

Single gene test and Sanger sequencing

Genetic testing has been a part of the diagnostic process for children with SNHL since 1997, when the *GJB2* was the first gene identified causing isolated HL[66]. In recent decades, there has been a shift from single-gene testing with Sanger sequencing to massively parallel sequencing (MPS). Sanger sequencing was invented in the mid-seventies by Fred Sanger[67,68]. In this method, the DNA sequence/gene of interest is fragmented by a chain-termination inhibitor, amplified by the polymerase chain reaction (PCR), and the position of the individual bases (A, T, C, and G) is identified using fluorescence and electrophoresis. In addition to identifying individual bases, MPS enable the entire exome or genome to be sequenced at the same time, and thus all potential genes related to SNHL can be sequenced simultaneously. Although MPS has become available (and affordable) in recent decades, Sanger sequencing is still cost-effective and fast, and the preferred method in some situations. This is the case, for example, when testing a specific well-known genetic variant to evaluate carrier status, in prenatal testing and segregation analysis, but sometimes also to confirm variants identified by MPS[69].

Genetic outcome prior to massive parallel sequencing

At the Department of Otorhinolaryngology at Skåne University Hospital

Before the introduction of MPS, genetic testing for children with non-syndromic SNHL was often limited to *GJB2*. Further genetic testing was conducted according to hereditary patterns, phenotype (i.e., symptomatology), and suspected syndromes[70].

We performed a retrospective study at the Department of Otorhinolaryngology at Skåne University Hospital to determine the diagnostic yield prior to the implementation of MPS at the end of 2018. During the period 2013 to 2017, 338 children who used conventional hearing aids were identified. Among these, a quarter (26%) underwent genetic testing and 18% received a genetic diagnosis (either based on a genetic test or family history), whereof 4% (n=13) had variants in *GJB2*.

During the same period (2013-2017), 46 children with severe SNHL were born and received cochlear implants (CIs) at the Department of Otorhinolaryngology at Skåne University Hospital, of whom 34 received bilateral CIs. Of these children 47% (n=21/46) were initially genetically tested with a single-gene test, and 20% (n=9/46) had PVs in *GJB2*.

Whole exome and whole genome sequencing

The whole human genome was sequenced for the first time in April 2003, an analysis that started in September 1990. Nowadays, genetic tests with MPS of the exome or the whole genome are part of clinical practice. In our study, DNA was extracted from a blood sample, but DNA can be extracted from other tissues in the body as well as from saliva.

Whole exome sequencing (WES) sequences the protein-coding part of the genome, while the entire DNA sequence, the whole genome, is sequenced using the whole genome sequencing (WGS) technique. DNA is fragmented into sequences (350-550 base pairs), with 150 base pairs paired at the beginning and the end of each sequence (TruSeq DNA PCR-Free). To obtain reliable data, the sequences are read several times and double-checked to ensure that the detected variants are not due to sequencing errors. This is referred to as read depth. In WGS, each nucleotide is read on average 30 times, a read depth or depth of coverage of 30x. In WES, the read depth is higher, and in mtDNA, the read depth is over 5000x[71]. In our projects, we used the Illumina NextSeq 500 (Illumina, USA) with an average read depth of 80x and the Illumina NovaSeq 6000 (Illumina, USA) with an average read depth of 30x, for WES and WGS, respectively.

Figure 5. The process of analysing genetic variation

After sequencing, the mapping process begins, where the reads (strings of DNA) are aligned to their correct position in the genome compared to a reference genome, Genome Reference Consortium Human Build (GRCh38)/Human genome build 38/(hg38)[72] (**Figure 6**). Interestingly, 70% of this reference genome is based on an anonymous male, and the remaining 30% comes from cell lines from around 60 people.

Once the sequences are aligned, the variation from the reference genome can be identified. As there are millions of variants, where less than 1% are relevant as disease causing[13], this process is aided by bioinformatic software. In our project, the in-house pipeline (GitHub - SMD-Bioinformatics-Lund/nextflow_wgs) for variant calling was used. The importance of the variants is estimated, among other factors, by allele frequency, consequences of the variant, prediction of the impact of the protein, and previous reports of pathogenicity. The variants are scored and ranked, and uploaded to Scout, the main manual interpretation tool. To interpret and determine the pathogenicity of the variants, the guidelines according to ACMG, discussed in the previous chapter, are used[17,18,73]. The variants are then annotated as being relevant for the symptomology or not (**Figure 5**).

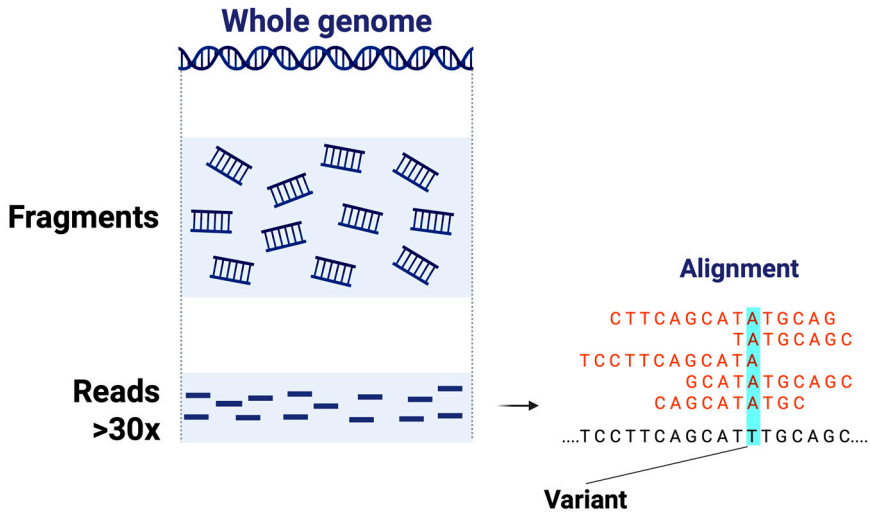


Figure 6. WGS sequencing, alignment of sequences
Created in BioRender. Elander, J. (2025) https://BioRender.com/acz9hww

Mitochondrial DNA sequencing

Although the mtDNA is a much smaller molecule, it is sequenced in the same way and can be processed together with the nDNA when doing WGS. In our pipeline, sequencing of mtDNA was added in May 2021. The method is validated for detection down to five percent heteroplasmy (variant allele frequency).

Comparing WGS and WES

There are advantages with both WGS and WES techniques, respectively, but when it comes to coverage and diagnostic yield, sequencing the whole genome is preferable[74,75]. Overall, WGS contributes to the more reliable detection of point mutations across the entire genome, whereas WES is efficient in detecting point mutations in the protein-coding part of the genome. Variants in the introns, detected with WGS, may affect regulatory mechanisms and hence the protein expression. Also, WGS enables a more efficient detection of CNV and other structural variants. Yet, when using WGS, the dataset is larger and more complex, and there is a higher risk of incidental findings unrelated to the primary purpose of the survey than when using WES. In fact, the DNA coding sequence part of the genome can be surveyed with nearly equivalent quality using WES as with WGS, although WGS covers the periphery of the exons is better. The diagnostic yield is higher with WGS for Mendelian disorders[76]. The reading depth of WES is higher[77]. Also, WES is more cost-effective[76].

To complicate it further, WGS can be performed as either short-read or long-read sequences, and there are advantages and limitations with both methods. With short reads, the accuracy of detecting small nucleotide variants (SNPs) and insertions/deletions is superior. However, structural variants, CNVs, and homologous repeats are more likely to be identified with long reads[78].

Gene panels

MPS provides a vast amount of data, and to make targeted analysis related to HL feasible, a gene panel is utilized. The number of genes on the HL gene panels differs between genetic laboratories, but the aim of the gene panel is the same: to improve clinical sensitivity and to avoid analysing gene variants with no clinical relevance for HL. The ACMG has defined clinical standards for creating a gene panel[79]. In their report, they state that for a gene panel to be cost-effective, it should include all genes associated with a Mendelian disease and the symptom, include genes with new evidence of pathogenicity, but limit or exclude genes of uncertain significance in relation to the investigated condition and thereby also limit the detection of VUS[79].

HearSeq

The gene panel used in our studies was developed by our co-researchers in the Department of Clinical Genetics in the Region of Skåne. This gene list focuses on isolated HL and non-syndromic HL mimics. In cases where a more complex symptomatology is evident, an open genome sequencing analysis of the proband and both parents, a so-called trio analysis, might be more efficient with a higher diagnostic yield and thus recommended[80,81].

The gene panel is updated regularly and cross-checked with updated genes related to SNHL reported in Genomics England Panel App, "a crowdsourcing tool to allow gene panels to be shared, downloaded, viewed, and evaluated by the Scientific Community"[69,82]. The current and all previous versions of the HearSeq gene panel are available at https://genpaneler.genetiklund.se, along with the gene numbers and a description of the associated phenotypes[83]. The genes included in version 9 (updated March 20, 2025) are presented in **Table 2**.

Table 2. Genes included in hearseq gene panel version 9

ABHD12	*CLRN1*	*GGPS1*	*LRTOMT*	*PBX1*	*SLC29A3*
ACTG1	*COCH*	*GIPC3*	*MAN2B1*	*PCDH15*	*SLC33A1*
ADGRV1	*COL11A1*	*GJB2*	*MANBA*	*PDZD7*	*SLC4A11*
AFG2A	*COL11A2*	*GJB6*	*MARVELD2*	*PEX1*	*SLC52A2*
AFG2B	*COL2A1*	*GNAI3*	*MASP1*	*PEX26*	*SLC52A3*
AIFM1	*COL4A3*	*GPR156*	*MGP*	*PEX6*	*SLITRK6*
ALMS1	*COL4A4*	*GPSM2*	*MINAR2*	*PJVK*	*SMAD4*
AMMECR1	*COL4A5*	*GREB1L*	*MITF*	*PKHD1L1*	*SMPX*
ANKH	*COL9A1*	*GRHL2*	*MN1*	*PLCB4*	*SNAI2*
AP1S1	*COL9A2*	*GRXCR1*	*MPZL2*	*PLS1*	*SOX10*
ARSG	*COL9A3*	*GRXCR2*	*MSRB3*	*PLXNB2*	*STRC*
ATP11A	*CRLS1*	*GSC*	*MT-RNR1*	*PNPT1*	*SUCLA2*
ATP1A3	*CRYM*	*GSDME*	*MT-TS1*	*POLR1C*	*SUCLG1*
ATP2B2	*DCAF17*	*HAAO*	*MYH14*	*POLR1D*	*SYNE4*
ATP6V1B1	*DHODH*	*HARS2*	*MYH9*	*POU3F4*	*TBC1D24*
ATP6V1B2	*DIAPH1*	*HGF*	*MYO15A*	*POU4F3*	*TCOF1*
BCS1L	*DMXL2*	*HOMER2*	*MYO3A*	*PRPS1*	*TECTA*
BMP4	*DNAJC3*	*HOXA2*	*MYO6*	*PSMC3*	*TFAP2A*
BSND	*DNMT1*	*HOXB1*	*MYO7A*	*PTPRQ*	*TIMM8A*
BTD	*DSPP*	*HSD17B4*	*NARS2*	*RDX*	*TMC1*
CABP2	*EDN3*	*HSPA9*	*NDP*	*RMND1*	*TMIE*
CACNA1D	*EDNRA*	*ILDR1*	*NEFL*	*RNF220*	*TMPRSS3*
CCDC50	*EDNRB*	*KARS1*	*NLRP12*	*RPS6KA3*	*TNC*
CDC14A	*EFTUD2*	*KCNE1*	*NLRP3*	*S1PR2*	*TPRN*
CDH23	*EPS8*	*KCNJ10*	*OGDHL*	*SALL1*	*TRIOBP*
CDKN1C	*EPS8L2*	*KCNJ16*	*OPA1*	*SALL4*	*TUBB4B*
CDT1	*ESPN*	*KCNQ1*	*ORC1*	*SEMA3E*	*TWNK*
CEACAM16	*ESRRB*	*KCNQ4*	*ORC4*	*SERAC1*	*USH1C*
CEP250	*EYA1*	*KDM6A*	*ORC6*	*SERPINB6*	*USH1G*
CEP78	*EYA4*	*KIT*	*OSBPL2*	*SF3B4*	*USH2A*
CHD7	*FDXR*	*KMT2D*	*OTOA*	*SGPL1*	*USP48*
CHSY1	*FGF10*	*LARS2*	*OTOF*	*SIX1*	*WBP2*
CIB2	*FGF3*	*LETM1*	*OTOG*	*SLC12A2*	*WFS1*
CISD2	*FGFR2*	*LHFPL5*	*OTOGL*	*SLC17A8*	*WHRN*
CLDN14	*FGFR3*	*LMX1A*	*P2RX2*	*SLC19A2*	
CLDN9	*FOXI1*	*LOXHD1*	*PAX2*	*SLC26A4*	
CLPP	*GATA3*	*LRP2*	*PAX3*	*SLC26A5*	

The difference between gene panels can be understood, at least in part, from the gene panel creator´s view on evidence. Whether there has been an inclusive or a more restrictive view of the genetic evidence for the link between the gene and HL. The more genes added to the panel, the more complex the analysis becomes. This may result in more VUSs in genes of uncertain significance, which may lead to

increased uncertainty and concern rather than an increased number of confirmed diagnoses[79]. On the other hand, with a too restrictive gene panel, there is a risk that interesting PVs will remain unidentified. The number of genes in the HL gene panel is also influenced by the number of syndrome-associated genes, where SNHL is part of the symptomatology, included.

Comparison between gene panels: OtoSCOPE® v9, Radboud DG 3.6.0, PanelApp v 4.22

For the clinician, it can be challenging to determine which laboratory to use and to comprehend the clinical differences between different sequencing methods and gene panels. At first glance, it might seem like the more genes on the list, the better. However, as discussed in the previous section, this is not always true. To gain understanding of the differences between some of the gene panels for HL, which have a high impact, I performed a comparison between gene panels. This allowed a reflection on how the choice of a specific gene list can influence the outcome in terms of genetic yield.

A SNHL gene panel often referred to is OtoSCOPE® v9, developed in Iowa, USA, with 224 genes. Another well-known gene panel orginates from a laboratory in Nijmegen, the Netherlands; Radboud DG 3.6.0. This gene panel is used by some Swedish departments for genetic diagnostics of HL. It is a WES hearing impairment panel with 254 genes. Another gene panel is the aforementioned PanelApp 4.22 from England. This panel focuses on non-syndromic HL and comprise of 147 genes and is curated regularly. When comparing these gene panels, I concluded that most of the genes on the lists are the same, but 27, 57, and 19 genes are unique on OtoSCOPE® v9, Redboud DG 3.6.0, and PanelApp 4.22, respectively.

When the above-mentioned gene panels were compared to the HearSeq v8 gene panel, the similarities were more obvious than the differences. Nevertheless, 22 of the genes on the HearSeq v8 are not included in any of the other lists, and 14 genes are only included in HearSeq v8 and PanelApp 4.22. Additionally, 116 genes are included in one or more of the other lists (**Figure 7**) but are not represented in the HearSeq list.

The genetic findings in our cohort of studied patients, discussed in the next chapters (Paper II and Paper III), would have been identified with all other gene panels except one finding, which would have been missed with PanelApp 4.22 (**Figure 7**). Whether more pathogenic variants would have been found with another gene panel than with HearSeq v8 has not been analysed and is therefore uncertain.

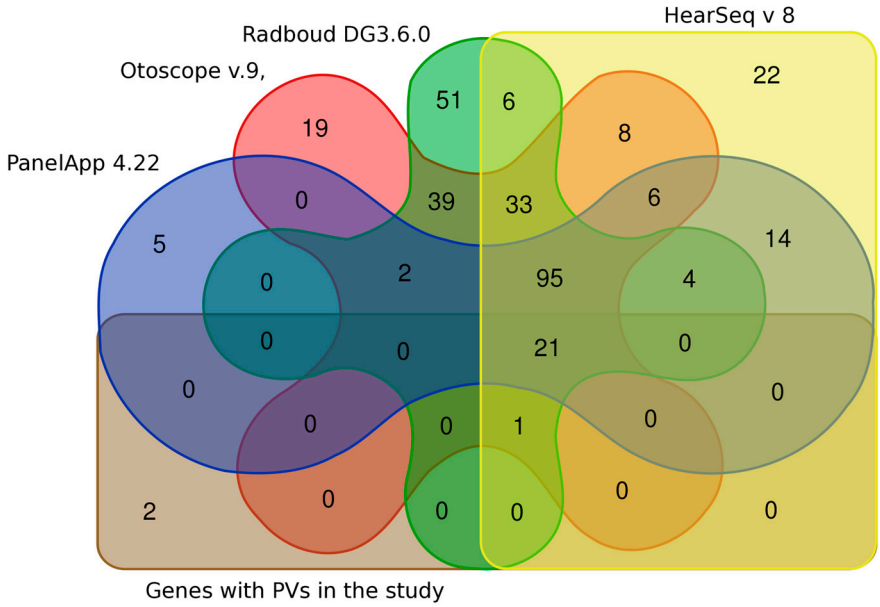


Figure 7. Comparison between different HL gene panels: PanelApp 4.22 (blue), OtoSCOPEÒ v9 (red), Radboud DG 3.6.0 (green), HearSeq v8 (yellow), and the genes with pathological genetic findings presented in Paper I and Paper II (brown).
Created by Elander, J. (2024) https://bioinformatics.psb.ugent.be/webtools/Venn

The main conclusion of this comparison was that the majority of pathogenic variants will be identified regardless of the chosen gene panel. Also, the number of genes included in the panel is not as important as the opportunity to discuss and receive support in interpreting the genetic results from a clinical geneticist. A genetic diagnosis of a disease requires a thorough description of the phenotype and close collaboration between the medical audiologist and the clinical genetic laboratory. A clinical geneticist is essential for a definite genetic diagnosis[81].

From single gene test to massive parallel sequencing

Implementation of massive parallel sequencing

The genetic heterogeneity in SNHL is the main rationale behind using MPS as a diagnostic method. Instead of starting the genetic diagnostics with a single gene test analysed by Sanger sequencing for *GJB2*, MPS analysis according to a gene panel is more efficient. Already in 2015, Shearer et al.[84] wrote that MPS should be considered as standard of care. Furthermore, in the algorithm of the American College of Medical Genetics and Genomics guideline for etiological background of SNHL from 2014[23], MPS was described as part of the investigation.

MPS was implemented as a clinical test for people with SNHL at the Departments of Otorhinolaryngology at Skåne University Hospital and Audiology at Örebro University Hospital, in collaboration with the Department of Clinical Genetics at Skåne University Hospital, in December 2018. Prior to testing, the study subjects or their parents (in cases of infants and small children) were offered to participate in a clinical study. The aim of this study was to describe the genetic variation in a Swedish population of individuals with SNHL. This had been done elsewhere in the world[3,4,26,33,85-89], but not in Sweden. The first eleven patients included in the study were examined with WES and were described in Paper I. In June 2020, the analytic platform at the clinical genetic laboratory changed to WGS, and thus the whole genomes of the following patients were sequenced.

Successful implementation of the new testing program required collaboration[90]. Collaboration was not limited to the parents, the audiology physician, and the specialist in clinical genetics. Additionally, other colleagues and specialists, who meet and care for children with SNHL became involved (**Figure 8**). For example, when genetic sequencing was performed at an early age, pathological variants in genes associated with Usher syndrome could be detected before the onset of visual symptoms. This influenced how these children were taken care of by the ophthalmologist. The information obtained from the genetic test may also influence the treatment targeted by the hearing rehabilitation team or the pediatric clinic.

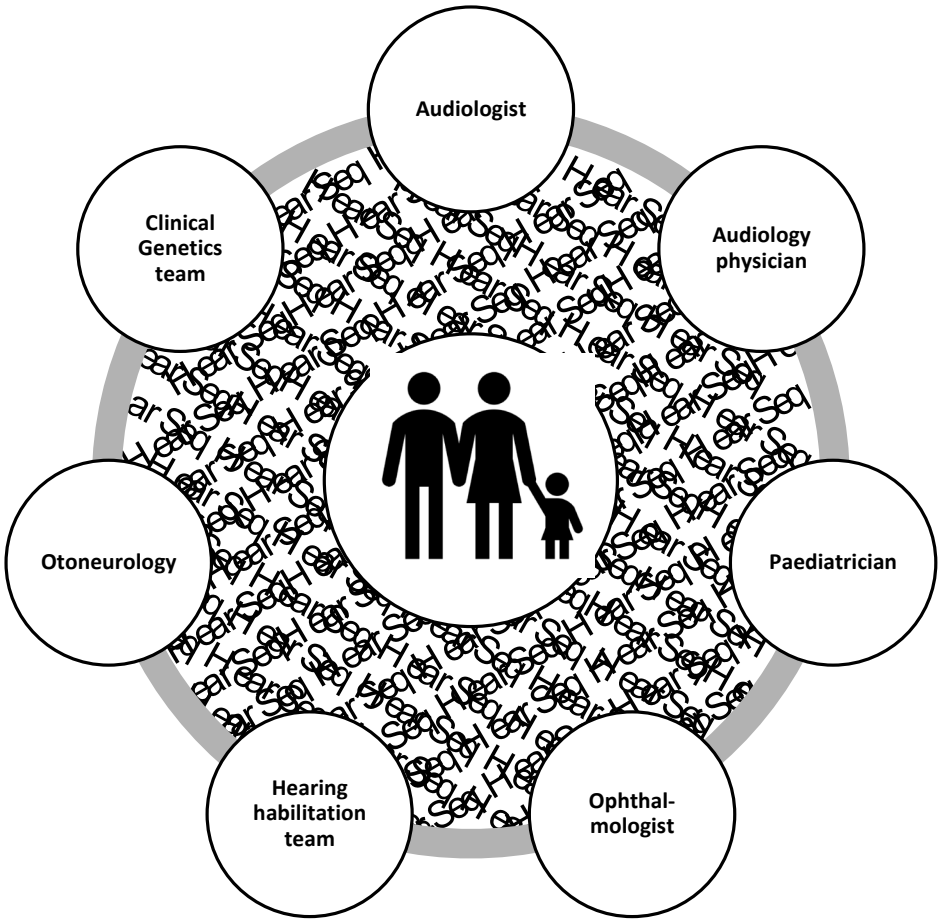


Figure 8. The implementation of WES/WGS required a multidisciplinary approach to the care of children with SNHL.

Diagnostic advantages with MPS

The advantages of a new and more advanced diagnostic testing modality are easy to recognize, both for researchers and physicians. Syndromic HL can be diagnosed prior to additional symptoms appearing, the prognosis for HL can be made more accurate, and rehabilitation efforts can be made more stringent.

Genetic treatment for children with SNHL

The value of identifying genetic pathological variation in the SNHL population is not limited to the benefits for the individual patient. Although there are currently no clinically available genetic treatment options for SNHL, the field is rapidly developing. Identifying the genetic variation in our population is crucial for the future implementation and development of genetic treatment options.

Genetic treatment of *OTOF*-related deafness

The major breakthrough in genetic SNHL therapy last year was the report from Fudan University, China, where *OTOF*-related deafness in humans was treated with a gene sequence delivered to the inner ear[91,92]. *OTOF* codes for Otoferlin, a synaptic protein, enabling exocytosis and replenishment of synaptic vesicles located in the pre-synaptic ribbons of the inner hair cells [93-95]. A deficiency of this protein is related to auditory neuropathy, as it reduces the efficiency of the transmission between inner hair cells and the spiral ganglion cells. In the study, they injected an *OTOF* sequence (using a dual-adeno-associated virus-1 vector with an inner ear-specific *MYO15*-promotor) through the round window membrane, while lifting the eardrum and visualizing the round window with an endoscope. Surgical approaches through the mastoid have also been described[96]. The viral vector was used to enable the sequence enter the cells through viral invasion. They reported that all but one had reduced ABR and ASSR thresholds from profound SNHL to mild-moderate HL (38-60dB). There is an ongoing study, still recruiting, with participating departments in Taiwan, the United Kingdom, and the United States[97]. The study uses the same technique, sponsored by Akouos Clinical Trials in Boston, which is also responsible for the study.

This revolutionary discovery has been an audiological goal in recent years, following the development of gene therapy for other diseases and advances in the audiological field. There are indications that an intact cochlear morphology enables use of genetic therapy for HL[98], and therefore, proteins that affect cell signaling, such as those involved in synapses, are a reasonable target for gene therapy. The fact that it has now been possible to install a gene sequence in the hardest bone of the body and thereby restore hearing in treated children is incredibly fascinating.

The clinical value of a genetic diagnosis

Parental experiences of single-gene testing

Since a genetic diagnosis, in most cases, does not lead to a specific treatment, it is reasonable to question whether the defined aetiology of SNHL provides added value for the patients and their families. Parental experience with single gene test, in this case *GJB2*, has previously been studied. According to Brunger et al.[99] parents saw genetic testing as positive and beneficial. Palmer et al. also found *GJB2/GJB6* testing beneficial, especially when the test result was positive, and concluded that the parents, at least, did not perceive the testing as harmful[100].

Models to evaluate genetic sequencing

The value of more extended genetic testing in children with rare diseases has been studied, and various models are used to evaluate genetic tests. At the beginning of the 2000s, the ACCE model, including analytic validity, clinical validity, clinical utility, and ethical, legal, and social implications, was developed[101]. The ACCE concept was further developed and used by the Evaluation of Genomic Applications in Practice and Prevention Initiative. This initiative was formed by the Office of Public Health Genomics at the Centers for Disease Control and Prevention in the United States, where genetic evidence was reviewed by an expert group[102].

The concept of clinical utility has been discussed, and different researchers have had slightly different definitions[103]. While some argue that clinical utility only refers to improved health outcomes[104], others argue that clinical utility also includes risks and benefits of genetic testing[105].

Nevertheless, clinical utility is related to whether the test improves health outcomes for the patient, while personal utility does not affect health or clinical care[106]. Bunnik et al.[106] argued that a genetic test must provide some form of useful information and have a purpose to achieve personal utility. A study by Hayeems et al.[107] further explored this concept and identified both intrinsic and instrumental uses for personal utility. The intrinsic use could be related to, for example, relief, whereas the

instrumental use was when the information could be useful to take some kind of action. Although they concluded that there is a personal benefit of WES/WGS in the investigation of rare diseases, it is not obvious that the same is true for isolated SNHL symptomatology. In a recent scoping review, Pezzullo et al.[108] studied indicators of genetic testing regardless of test cause or context. Among other things, they found that the evaluation of genetic tests is often insufficient regarding clinical efficacy and direct patient health outcomes.

In conclusion, when the WES study at the Departments of Otorhinolaryngology in Lund and Audiology in Örebro started in 2018, there was a knowledge gap in the literature about the value of extended genetic testing in children with SNHL. Information about the parental experience of children with SNHL who had undergone WES or WGS was limited. To evaluate the new test regime, a patient questionnaire was developed.

Questionnaire development

There was no suitable validated questionnaire on WES/WGS in children with SNHL. A questionnaire on patient-related experiences measures (PREM) developed by Karin Svensson, genetic counsellor at the Department of Clinical Genetics at Skåne University Hospital, was used. This was a questionnaire in Swedish that had been used to evaluate quality and patient satisfaction at the Department of Clinical Genetics. This questionnaire was based on two validated questionnaires, one patient questionnaire from the Swedish Association of Local Authorities and Regions and the other from the Regional Cancer Centres in Sweden. However, the questionnaire was not validated in its entire format. The questionnaire included 28 questions regarding demographic data, genetic information, monitoring and control program, accessibility, attendance and participation, support and needs, experience of the Department of Clinical Genetics, and genetic testing.

The questionnaire was adjusted for our purpose. The original questionnaire contained 28 questions in Swedish. The modified version contained 21 questions in Swedish, and 16 of these questions remained unchanged from the original. Twelve of the questions from the original questionnaire were assessed as irrelevant for patients with SNHL and removed. Five additional questions were added. The modified PREM questionnaire concerned information, follow-up, availability, care, and participation, as well as personal experience of the test. Most questions in the questionnaire were based on a Likert scale with four alternatives of answers (from strongly agree to disagree). A Likert scale is a commonly used ordinal scale where the response options usually vary between four and seven[109]. However, a questionnaire with Likert-type questions is more reliable with an increased number of response options on the scale[110]. In addition, care must be taken in how the data

is presented, as there is no linear relationship between the optional responses[111]. Three questions had open-ended responses, and one question had multiple-choice options. The following is a translated version (from Swedish to English) of the questionnaire used in Paper II (**Table 3**). The original questionnaire in Swedish is available as a supplement (**Supplement table 1**).

Table 3. English version of the questionnaire used in the pilot study for parents whose children had been genetically tested.

FOLLOW-UP AFTER THE STUDY OF GENETIC CAUSES OF HEARING LOSS				
Please circle the answer that best reflects your level of agreement				
1	I was asked and received information about participating in a study on genetic testing for hearing loss			
	STRONGLY AGREE	AGREE	SOMEWHAT DISAGREE	DISAGREE
2	The testing found that the hearing loss had a genetic cause			
	YES	NO	*(If No, please skip to question 19)*	
TESTING/INFORMATION				
3	My knowledge of the genetic condition improved			
	STRONGLY AGREE	AGREE	SOMEWHAT DISAGREE	DISAGREE
4	I understand how to communicate knowledge/information about the genetic condition to my relatives			
	STRONGLY AGREE	AGREE	SOMEWHAT DISAGREE	DISAGREE
5	I received information *(Please indicate the item(s) that best match your situation)*			
	IN AN OUTPATIENT CONSULTATION	BY WRITTEN LETTER OR E-MAIL	OVER A PHONE CALL	INFORMATION LEAFLET
	ANOTHER WAY	I DID NOT RECEIVE ANY INFORMATION *(if yes, please skip to question 9)*		
6	The spoken information was clear			
	STRONGLY AGREE	AGREE	SOMEWHAT DISAGREE	DISAGREE
7	The written information was clear			
	STRONGLY AGREE	AGREE	SOMEWHAT DISAGREE	DISAGREE
8	I wish I had received information in the following way			
FOLLOW-UP				
9	I was informed about what kind of follow-up is important for my child			
	STRONGLY AGREE	AGREE	SOMEWHAT DISAGREE	DISAGREE
10	I trust the medical assessment that was made			
	STRONGLY AGREE	AGREE	SOMEWHAT DISAGREE	DISAGREE
AVAILABILITY				
11	It was easy to contact the outpatient clinic			
	STRONGLY AGREE	AGREE	SOMEWHAT DISAGREE	DISAGREE
12	My preferences for appointment times to the outpatient clinic were considered			
	STRONGLY AGREE	AGREE	SOMEWHAT DISAGREE	DISAGREE
13	Do you have any opinions on availability during the genetic testing, such as appointment letters, phone appointments, waiting times, travel routes, or anything else?			

CARE AND PARTICIPATION				
14	I was treated with respect			
	STRONGLY AGREE	AGREE	SOMEWHAT DISAGREE	DISAGREE
15	Those I encountered or had contact with understood my situation			
	STRONGLY AGREE	AGREE	SOMEWHAT DISAGREE	DISAGREE
16	I understand that information about me, my family and my relatives is important for the genetic testing			
	STRONGLY AGREE	AGREE	SOMEWHAT DISAGREE	DISAGREE
17	My experiences and knowledge of the genetic condition were asked for			
	STRONGLY AGREE	AGREE	SOMEWHAT DISAGREE	DISAGREE
18	My questions were answered:			
	STRONGLY AGREE	AGREE	SOMEWHAT DISAGREE	DISAGREE
PERSONAL EXPERIENCE OF THE TEST				
19	I feel that the genetic testing provided additional value for me and my family			
	STRONGLY AGREE	AGREE	SOMEWHAT DISAGREE	DISAGREE
20	I would recommend other families with children with hearing loss to go through this type of testing:			
	STRONGLY AGREE	AGREE	SOMEWHAT DISAGREE	DISAGREE
21	Do you have additional thoughts/ideas/reflections regarding the genetic testing?			

This questionnaire–with occasional changes and amendments–is based on a questionnaire used in the study "EVALUATION OF PATIENT EXPERIENCE OF QUALITY AND SATISFACTION ASSOCIATED WITH CLINICAL GENETICS OUTPATIENT SERVICES" by Karin Svensson, Genetic counselor, Clinical Genetics, Lund, SUS. Permission to use the questionnaire has been obtained from the author.

Questionnaire validation process

The questionnaire was revised to be suitable for our research questions, but has not been properly validated. It is therefore uncertain whether the questionnaire measures what it is intended to measure (valid) or whether the measurements are consistent (reliable). A stepwise validation process can be conducted following different frameworks[112], where both validity and reliability need to be assessed from different angles. Nevertheless, the questionnaire was used in Paper II as a pilot study. Further assessment of validity and reliability is needed to develope a robust quantitative research investigation tool for parental experiences of genetic testing related to the HL of their children.

Paper II and Paper III

Papers II and III are two prospective studies on pathogenic variation in patients with SNHL examined at two tertiary audiology units: the Department of Otorhinolaryngology, Skåne University Hospital, Sweden, and the Department of Audiology, Örebro University Hospital, Sweden. The inclusion criteria were bilateral SNHL threshold of ≥25 dB HL. Children were the main focus of the study, but teenagers and adults could also be tested if a genetic cause was suspected. In Study II (December 2018 to June 2020), sequencing was performed using WES, whereas in Study III (July 2020 to December 2022), it was performed using WGS. When this new sequencing method was initiated in a study form, children with severe SNHL were prioritized in the clinic by the doctors. This explains why all patients in study II had severe SNHL. Patients who had previously undergone testing with *GJB2* without a genetic finding were not excluded. Since the two studies shared the same primary purpose, namely, to describe genetic variation in a population with HL in Sweden, the results and discussion are presented in part together.

Aim

Paper II:

Describe the genetic variation in Swedish children with profound SNHL.

Evaluate the family experience and describe the process of implementation of WES.

Paper III:

Describe the genetic variation in a Swedish population with mild to profound SNHL.

Identify factors relevant for a higher diagnostic yield.

Methods

Paper II:

The HL was diagnosed by a standard age-appropriate audiological evaluation. All the tested children in this study were candidates for CIs. Their DNA was extracted

from venous blood and examined with WES. A HL gene panel with 179 genes was used.

The follow-up questionnaire (**Table 3**) was distributed to the parents, and a reminder was sent out when the questionnaires had not been returned. In six cases, the questionnaires were translated orally to the parents, three to English during a clinic visit, and three by an interpreter.

Paper III:

The HL was diagnosed by a standard age-appropriate audiological evaluation. The SNHL was classified as mild (21-40 dB HL), moderate (41-60dB HL), severe (61-80 dB HL), or profound (>80dB HL). DNA from patients with SNHL was extracted from venous blood and examined with WGS. The HearSeq gene panel was used.

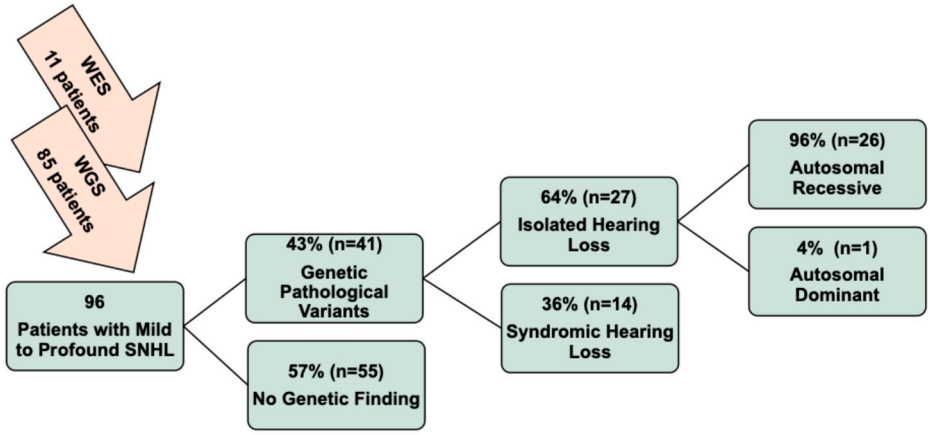
Descriptive statistics, including the diagnostic yield in the SNHL severity groups, were analysed. The diagnostic yield in the different groups, as well as time of onset of SNHL (divided into prelingual (<2 years) and postlingual), was analysed with the Chi-Square test to identify associations between the groups. Additionally, the subgroups were separately compared with Fisher´s exact test. Also, multinomial logistic regression analysis with profound SNHL as the reference was performed

Results

Table 4. Basic demographic features in Papers II and III

		PAPER II: WES	PAPER III: WGS
Female/male (n)		7/4	51/34
Age, median, range (years)		2.5, 0.4-11	6.75, 0.2-73
SNHL	Mild n (%) Moderate n (%) Severe n (%) Profound n (%)	 11 (100%)	12 (14%) 24 (28%) 9 (11%) 40 (47%)
SNHL	Prelingual n (%) Post Lingual n (%)	11 (100%) 0	57 (67%) 28 (33%)
Genetic yield n (%)		3 (27%)	38 (45%)

The two studies differ in terms of the characteristics of HL, as all participants in the WES study had severe SNHL and were candidates for CI, while age, degree, and time of onset of SNHL varied more in the WGS study (**Table 4**). However, in this section, the genetic results from Study II and Study III have been grouped together to provide a clearer picture of the pathogenic variation (**Flowchart 2**).



Flowchart 2. Diagnostic yield in the WES (paper II) and the WGS study (paper III).

The diagnostic yield includes only variants classified as likely pathogenic (ACMG 4) or pathogenic (ACMG 5), which are regarded as solved cases and henceforth described as PVs. In our material, some VUS were identified. If VUS were identified in Usher-associated genes, the genetic finding would lead to a complementary examination by an ophthalmologist and ERG. Identification of complementary symptoms can provide stronger evidence of pathogenicity, potentially leading to the reclassification of variants. None of the identified VUS were reclassified based on the clinical examination.

PVs were identified in 25 different genes (**Figure 9**). Most of the PVs were associated with isolated SNHL. Syndromic SNHL was identified in 37% (n=15) of the cases.

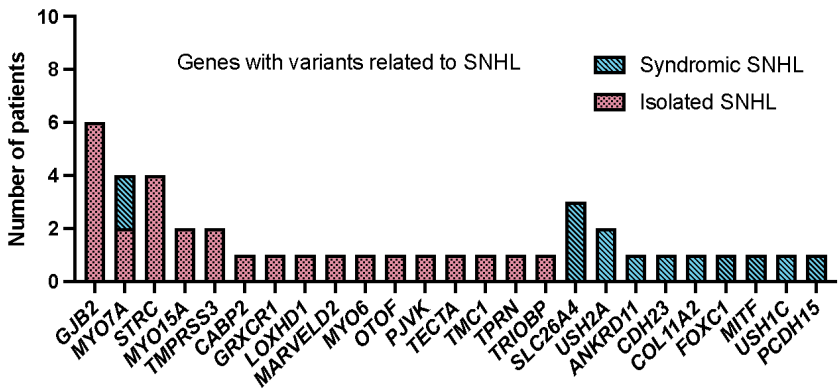


Figure 9. Genes (n=25) with pathogenic variants related in Studies II and III

The total diagnostic yield in the two sequencing studies was 43% (n=41/96). In moderate to profound SNHL, the diagnostic yield (WES + WGS) was 48% (n=40/84). The diagnostic yield was higher among patients with a prelingual onset of SNHL 52% (n=35/68). In the group with mild SNHL, one (8% n=1/12) had a verified genetic diagnosis (**Figure 10**). The HL in this case was progressive, and follow-up audiological tests revealed moderate SNHL.

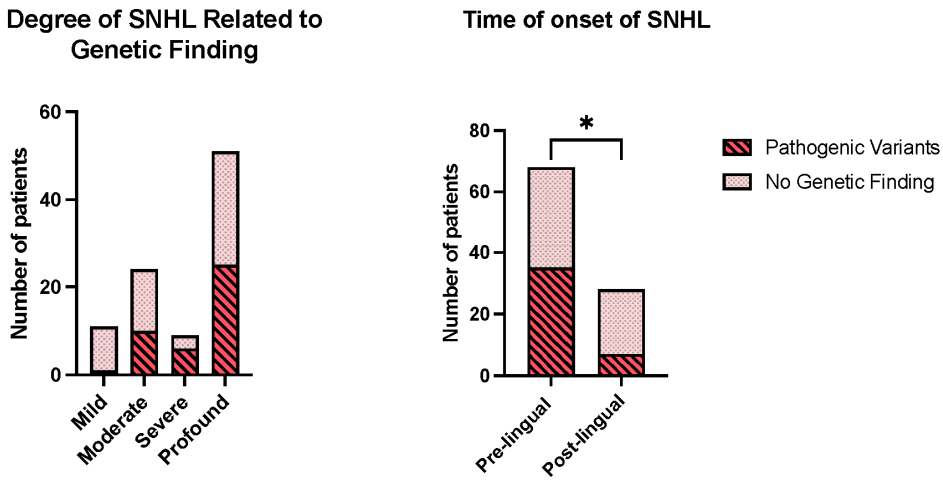


Figure 10. Yield Related to Degree and Onset of SNHL in Papers II and III. Fisher Exact test *p=0.02.

Among the patients with SNHL, 43 patients (45%) had parents who were born in a country other than Sweden. The genetic variation in the population, therefore, did not solely reflect a Swedish genetic background. Twenty-nine participants had parents who were born in the Middle East. The inheritance pattern for PVs also differed between the groups, with a higher diagnostic yield (58%, n = 17/29) and mainly autosomal recessive homozygous inheritance patterns in the group originating from the Middle East (**Figure 11**). In the group with homozygous PV originating from the Middle East, the parents of 10 of the children self-reported that they were relatives.

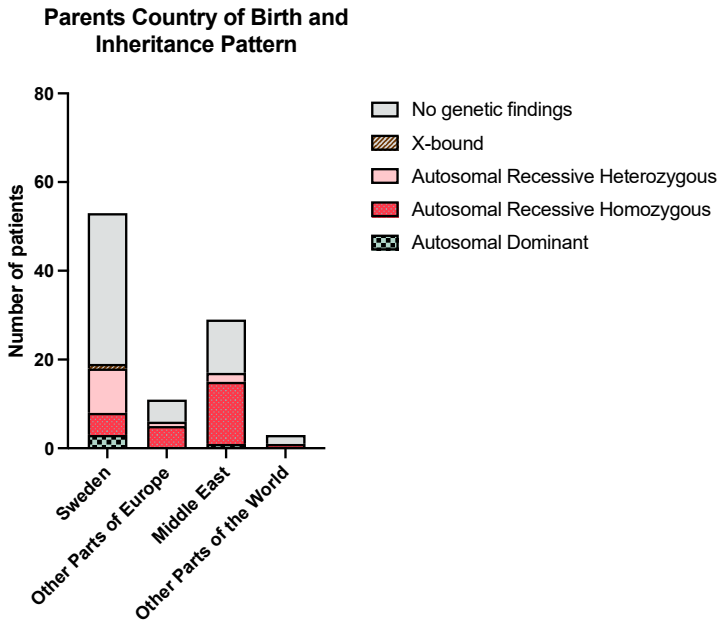


Figure 11. Inheritance pattern and the country of birth of the parents.

Paper II: Pilot questionnaire study

Parents of all eleven children in the WES study completed the questionnaire. Question numbers 3-18 were answered by parents for five of the children. They had received a PV´s answer or got information about a VUS, resulting in complementing eye examinations. Overall, the responses were positive, where four of the parents responding agree or strongly agree to the categories: information, follow-up, availability, care, and participation. Nevertheless, there was one exception, where parents of one child got information by mail, and they responded with "somewhat disagree" and "disagree" on all but two questions. These parents added in the last open-ended question that they had a wish for a verbal conversation and expressed it as follows. "Would like to talk to an expert to get more information/knowledge. Received other information in my home country about the cause of the hearing loss (vaccination at age three?)".

On questions 19 and 20, about personal experience of the test, the response was positive. All but one participant felt that the genetic test added value for the family, and all participants recommended the genetic test for other families in the same situation (**Figure 12**).

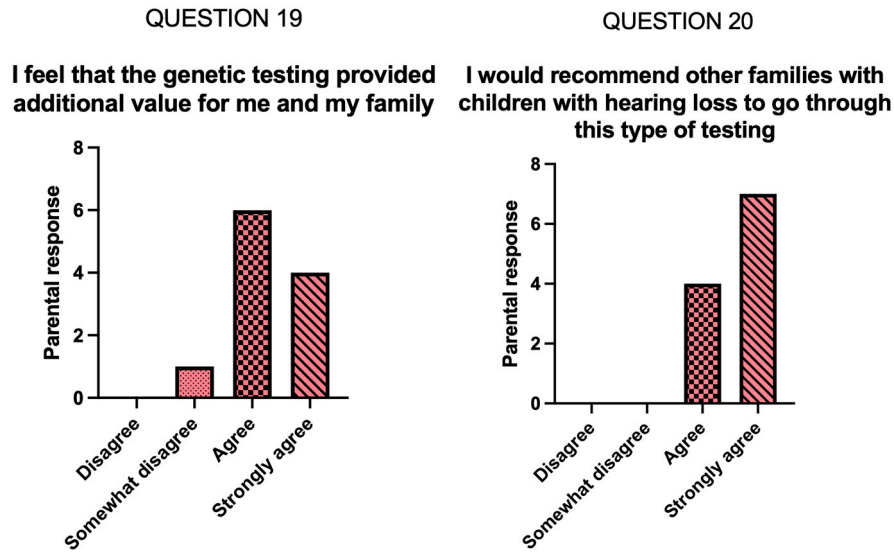


Figure 12. Number of answers on Likert scale alternatives on questions 19 and 20.

Discussion

These two studies described for the first time the genetic variation in a Scandinavian population with HL. The genetic yield of 43% was consistent with what has been previously shown in other parts of the world[26,30,33,85,89,113]. PVs were identified in 25 different causative genes.

Genetic SNHL related to PVs in *GJB2* (n=6/96, 6.3%) was identified in fewer cases than expected[27,28]. This may be partly due to selection bias, as older patients may have previously been tested with the *GJB2* single-gene test.

Usher syndrome was the most common syndromic SNHL, diagnosed in 8 cases, with PVs found in 5 different causative genes (*MYO7A, USH1C*, *PCDH1, CDH23, USH2A)*. Additionally, three PVs were identified in *MYO7A*, and these patients were referred to an ophthalmologist for evaluation of visual function. In these three cases, the retinal status was assessed as normal with ERG. Since the prevalence of Usher syndrome was slightly higher in our cohort than expected[37], the question arose whether Usher syndrome has been previously underdiagnosed.

The fact that a shared family background accumulates autosomal recessive traits is a well-known phenomenon that is not only familiar to professionals. However, this knowledge does not influence behaviour within certain subcultures. A study from Belgium[89] had similar findings both regarding ethnicity and diagnostic yield, compared to our study. They found, that nearly 40% of the included patients with

SNHL had non-European origin, and a definite genetic diagnosis was more common in the non-European group. In a study from Saudi Arabia, a population where consanguinity is common, the parents were related in 83% of cases of hereditary SNHL[114], and the prevalence of SNHL was ten times higher in the Saudi Arabian population than in Western countries[115].

Paper III included patients with mild SNHL. In this group, only one of twelve patients received a genetic diagnosis. Although there may be an identifiable genetic cause in patients with mild SNHL, the relevance of testing is questionable. It is doubtful whether a genetically identified cause of mild SNHL has clinical utility in terms of improved health outcomes. Also, on a personal level, the benefits of genetic testing are reasonably limited.

Positive response to the pilot questionnaire in Paper II

Despite the insufficient validation of the questionnaire, the pilot study laid the foundation for insights and meaningful findings regarding parental experiences of genetic sequencing. The study revealed a tendency toward positive attitudes among parents, as all parents of the eleven children tested in the WES study recommended other families in the same situation to take the test. Nevertheless, there was a need to examine the experiences of testing to determine the relevance of the questions asked.

The personal value of genetic testing related to SNHL will be discussed further in relation to Paper IV, a qualitative thematic analysis of parental experience of genetic testing.

Qualitative research –to explore and understand

The genetic code is only part of the picture

Since genetic testing has a limited impact on treatment, as in the case of genetic testing for SNHL, it is not obvious that genetic testing and results add any value for the patient. Therefore, the testing may be questioned. There are potential drawbacks to genetic tests, aside from the cost of genetic sequencing. Some drawbacks are mentioned below.

- A negative genetic test does not rule out a genetic etiology.
- Around half of the patients remain undiagnosed, which can lead to frustration and misunderstandings.
- There is a risk that patients receive unwanted information or incidental findings.
- There are no guarantees that genetic information or knowledge will be perceived as an asset by the patient.

Given these uncertainties, the value of WES/WGS for patients with SNHL and their families must be understood when genetic testing is offered. In a study by Tutty et al.[116] based on an Australian cohort of children with SNHL[33], parents responded to open-ended questionnaires (n=67) about their experiences with genetic testing. The responses were analysed using content analysis, and it was concluded that the tests provided certainty, led to empowerment, and a feeling that the tests were conducted in the best interests of their children[116]. In another questionnaire study, Cejas et al (2024)[117] investigated the parental experiences and barriers to genetic testing in 146 parents of children with SNHL. They were recruited as a convenience sample, mainly from social media platforms. Although less than half of the children of the participating parents had undergone genetic testing, the parents reported generally positive feelings, including excitement (64.4%), hopefulness (41.1%), and enthusiasm (28.1%) about new genetic discoveries.

As the literature in this field is limited, parental experiences with genetic sequencing of children with SNHL were crucial to further explore in order to understand the

potential value of WES/WGS. This experience is difficult to measure or randomize, and there are no dichotomous answers. Exploring of parental attitudes toward and experiences with genetic testing is important and can be achieved through qualitative methodology. Not only to understand how parents value genetic testing, but also because parents play an important role in the rehabilitation of the child and in their contact with healthcare providers. This background can be regarded as an argument for the study, the conceptual framework. The conceptual framework is the basis or rationale for conducting the study[118].

A qualitative method takes a different approach to research. It is appropriate at a knowledge-theoretical and epistemological level when exploring experiences that have not been previously investigated. Qualitative methods enable new discoveries and are suitable when there is limited prior research in the field. These results can then be triangulated with quantitative measurements. In fact, qualitative and quantitative methods complement each other and depend on the research question. The theoretical scientific background to qualitative research is briefly explained in the next section.

The nature of knowledge

For scientists, the nature of knowledge is essential. There are two fundamental concepts to describe knowledge. *Ontology* is defined as the study of being -how things are, whereas *epistemology* is the theory of knowledge, how we gather and view knowledge[119]. Even for a researcher trained in medical science, it is important to understand the ontological and epistemological background on which medical knowledge is based.

In medicine, research is usually based on a positivist approach or paradigm[120]. The positivistic paradigm has the assumptions that external reality exists, and that this reality can be understood by objective measures[118,121]. On the contrary, within the interpretative, also called the constructivist, paradigm[119], reality is dependent on social constructions, contexts, and experiences[122]. In this approach, reality is subjective, and researchers are more like explorers or adventurers seeking to discover and see patterns (**Figure 13**).

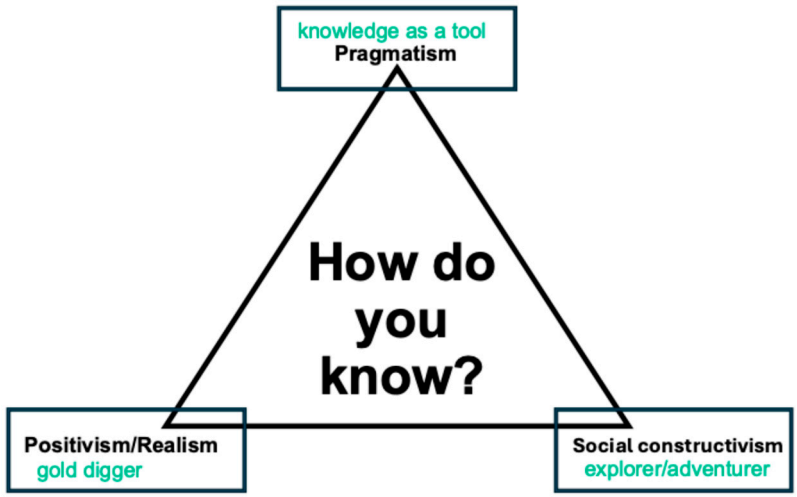


Figure 13. Epistemology and the nature of knowledge. The knowledge is dependent on the paradigm.

A paradigm is our understanding of the world and the knowledge of what we consider to be normal science. However, a paradigm can also unite a group of practitioners and their research[123], and be based on different views of knowledge. Thomas Kuhn was the first to describe the process of acquiring new knowledge as not linear. Instead, repeated anomalies or new discoveries that change our view of the world can lead to a scientific revolution or a paradigm shift[123].

Within the interpretative paradigm, there are various methodological approaches, including hermeneutic, phenomenology, social constructivism, and ethnography[124]. The uniting elements of all these methodologies are the importance of subjectivity, the relevance of the context, and interpersonal relations. Both hermeneutics and phenomenology focus on lived experiences. However, hermeneutic research is more interpretative, seeking a historical meaning, whereas phenomenology focuses on descriptions and structures to find meaning[121]. A phenomenological method is appropriate for studying a distinct phenomenon and lived experiences. Unlike ethnography, for example, where the cultural context is central, the focus is on the phenomenon itself.

This overview provides theoretical guidance ranging from the paradigm level to the methodology. Methodology is related to, but not the same as, method. When choosing the appropriate method for a study, it is essential to have an understanding of the methodology at a higher theoretical level. The choice of method depends on the research question and also on the experience and preferences of the researcher.

Data collection

Qualitative data can be collected from observations, images, video recordings, text from newspapers or websites, individual interviews, or group interviews. Interviews, either individual or in groups, are most common, at least in the phenomenological field.

Thematic analysis

There are various methods that can be used in qualitative research. Thematic analysis is a flexible qualitative method for structuring and analysing patterns in collected data and for identifying themes[125]. These skills are useful in many qualitative methods, and Braun and Clarke[125] argue that this method should be one of the first to learn by a qualitative researcher. Thematic analysis can be used to analyse lived experiences using a phenomenological methodology.

For qualitative research to give meaningful results, a rigorous method is crucial and should be transparent. Thematic analysis is a stepwise process to handle the collected data. Attride-Sterling[126] described a six-step process, including coding the material, identifying themes, constructing thematic networks, describing and exploring thematic networks, summarizing thematic networks, and interpreting patterns. The themes should be organized into basic themes, organizing themes, and global themes (**Figure 14**). A similar process is described by Braun and Clark[125], but they emphasise that, during the process, the themes should be reviewed. Their six steps include familiarizing yourself with the data, generating initial codes, searching for themes, reviewing themes, defining and naming themes, and producing the report. Themes are marked with different levels called codes, different levels of sub-themes, and finally organized into main themes. It is therefore the same type of thematic network, but with different vocabulary. In this thesis, basic themes, organizing themes, and global themes have been used as terminology. Other researchers have refined the process further. Skovdal and Cornish[127] describe the analytic process from data to final report in thirteen steps, whereas Kiger and Varpio[128] use the original steps from Braun and Clark[125] but provide a thorough description of each step.

In summary, be familiar with your data, ensure that the themes are truly based on the codes, and that the themes and thematic networks have been thoroughly reviewed. Throughout the analysis process, themes should be verified against the quotes. This is an empirical method to ensure that interpretations of meanings and themes are actually based on the statements of the informants. Therefore, it is a method that results in many quotes. The analysis should correlate well with the themes. It is essential to have a clear understanding of the method to avoid mistakes

during the process. Some basic things to be aware of are that subjectivity is rather an asset than a drawback. Themes do not emerge from the data; rather, the identification of themes is an active process carried out by the researcher. A common mistake is to categorize by topics rather than identifying themes. The problem is that codes organized according to a topic do not necessarily have a common meaning[129].

Figure 14. Thematic network

Deductive versus inductive

When using thematic analysis, this can be done from the bottom up or from the top down, using an inductive or deductive approach. An inductive approach can be described as data-driven. This means that the codes build up themes and, during the coding process, the researcher tries not to influence the themes that are identified. An inductive method is useful in unexplored fields of research for building a theoretical understanding of phenomena and this is the method chosen for the work in this thesis. A deductive approach is concept-driven; the analytical interest of the researcher influences the identified themes[125,130,131].

Quality in qualitative research

Qualitative research is subjective, and the primary research instrument is the researcher[129,131,132]. This contrasts with a positivist approach, where objectivity and the two concepts of validity and reliability are crucial to quality. Given these differences, several criteria are necessary for achieving high-quality studies in the field of qualitative research. Among others, Standards for Reporting Qualitative Research (SRQR) by O´Brien et al.[133] is commonly referenced.

For thematic analysis to achieve trustworthiness, the study should be based on credibility, transferability, dependability, and confirmability[131]. This means that for the research result to be trustworthy, the experiences need to be recognizable by the respondents and be able to be generalized or transferred to other contexts. Dependability refers to a logical and traceable research process, while confirmability is linked to the interpretations and analysis[131].

Paper IV

A qualitative study of in-depth interviews with parents of children with SNHL was examined with WGS

Introduction

Advancements in genetics have enabled the identification of PVs associated with HL. However, the significance of these findings for patients and their families remains uncertain. It is necessary to evaluate the utility and value of conducting genetic testing in this context.

Aim

To investigate the value of genetic tests in children with SNHL. This study explores the experiences of parents whose children with SNHL have undergone WGS. Additionally, the analysis identifies the associated benefits and risks.

Method

In-depth interviews were conducted with parents of children with SNHL who were genetically tested at the Departments of Otorhinolaryngology at the University Hospital in Lund and Audiology at the University Hospital in Örebro. Twenty parents of the last consecutively tested children were informed of the study by an information letter and then contacted by a phone call. The inclusion criteria were parents of children ≤5 years, and the interview could be conducted in Swedish without an interpreter. Ten parents (3 fathers, and 7 mothers) of nine of the children gave oral and written consent to participate in the study. A semi-structured interview guide was used with questions related to the topics in **Figure 16**, and the interviews were recorded with a voice recorder. Three interviews were conducted on the digital platform Zoom.

The audio files were manually transcribed. The resulting transcripts were coded and analysed using a reflexive thematic analysis approach, employing a stepwise process without a preexisting codebook, as described in previous chapter. The analytic process was inductive, where the essence of the content was coded and formed the basis for the basic themes.

Interview guide

The interview guide was designed to investigate how parents experience genetic testing both before and after the test was performed. The questions were related to information, expectations, and feelings before and after testing. There were also questions regarding how the genetic test results influence the family today. Additionally, expectations for the future and ethical considerations related to the test result were asked for. Questions and follow-up questions in seven different categories (**Figure 15**) were prepared. All the interviews covered the areas in the interview guide, but questions were adapted to explore the experiences and stories of each parent. The entire interview guide, translated from Swedish to English, is supplemented (Supplement table 2).

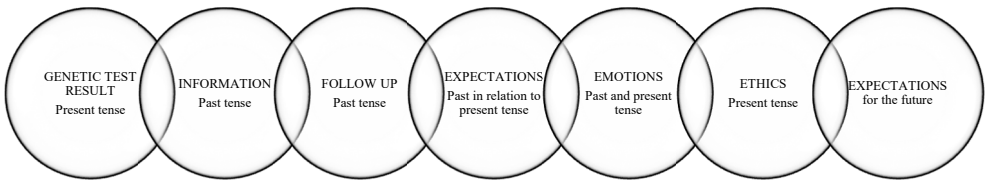


Figure 15. Areas of interest in the semi-structured interview guide.

Analytic process

The interviews were regarded as containing rich data, detailed descriptions, and elaborations on follow-up questions, and in the last interviews, no new themes were identified, and thus the study was regarded as saturated.

The analytic steps were followed. The interviewer, who also transcribed the material (JE), was familiar with the data, and a senior researcher read all the entire interviews for familiarisation. The analytic steps (**Figure 16**) were then followed by coding the transcripts and searching for themes by both researchers independently. This enabled co-judging in each step of the analytic process. The codes and themes were then cross-checked and the researchers were to a large extent congruent in their analysis.

Figure 16. The stepwise process of thematic analysis

The initial global themes identified during the third step are presented in **Figure 17**, to make the analytic process traceable.

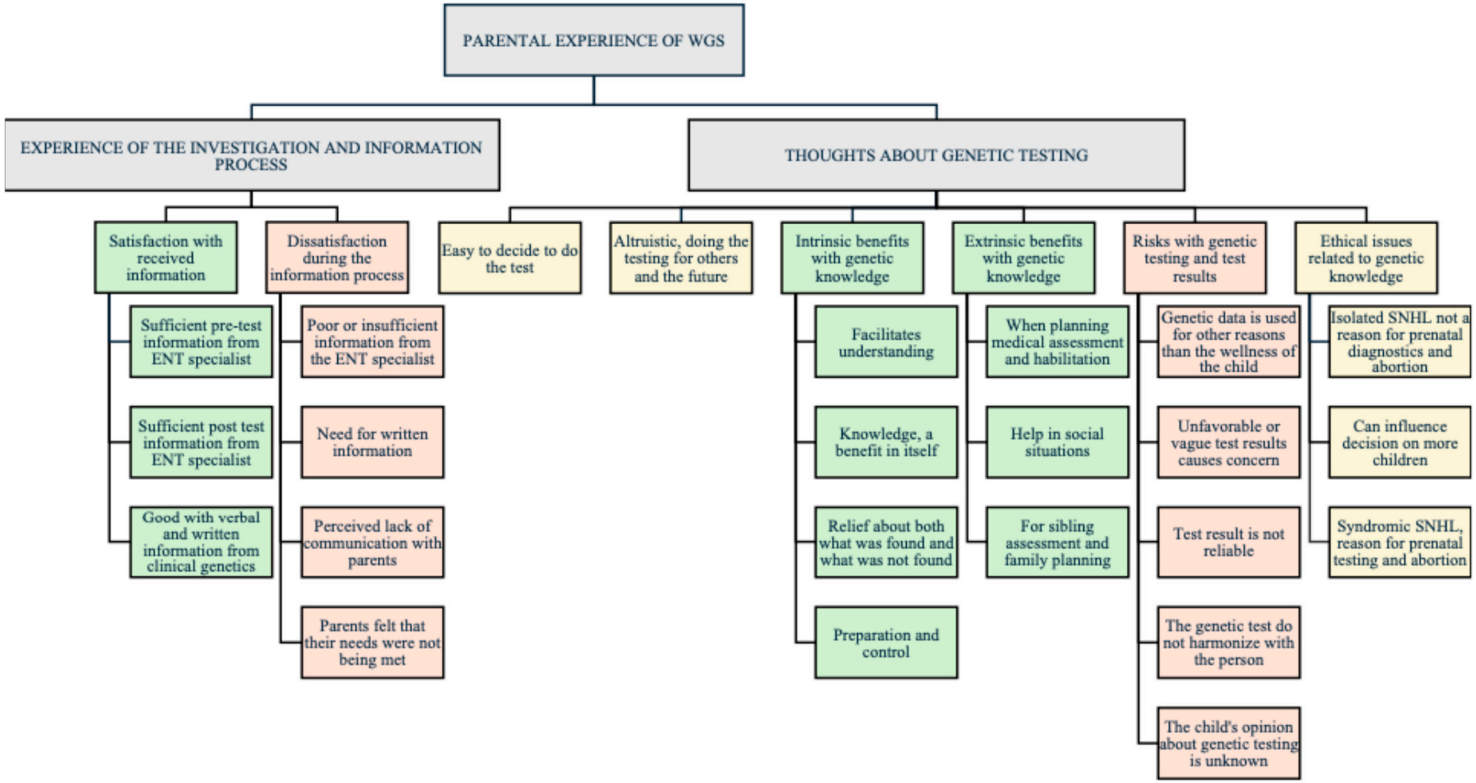


Figure 17. The initial global themes that turned out to be topics.

When reviewing the themes, it was realized that the initial global themes did not have a common meaning and were, in fact, topics. This is an example of the importance of reviewing the themes and following the stepwise process to avoid a potential pitfall with topics described by Braun and Clarke[129]. The basic themes were still reflecting the content of the quotes, but the global themes lacked a shared meaning or a central concept. Then, the basic themes were reanalysed and the organizing and global themes reviewed. Not all quotes are presented in the article. To get a deeper understanding of the data and to be able to follow the analytic process, selected quotes related to all the basic themes, are presented in the tables below, and also the themes on different levels (**Tables 5-7**).

Table 5. Quotes and basic and organizing themes in the first global theme

GLOBAL THEME 1 – LIMITED KNOWLEDGE CREATES UNCERTAINTY		
Organizing themes	**Basic themes**	**Quotes (study participant)**
Parents need of information was not being met	Wish for written information	*"Absolutely, you would like to have a written answer and maybe a little bit based on what we have discussed or said."* (# 4) *"Yes, I would need to have everything in writing."* (# 6)
	Lack of communication	A mother when receiving genetic test result about her child with Usher syndrome without any preparation. *"It was horrible. It was horrible. Because we didn't even know about it. We got a summons to the ENT specialist and that's about the only thing I can be really angry about, or not angry, but I don't know how they could have done it any better either. We got a piece of paper, and we thought we'll go to the ENT doctor and make a regular visit. We didn't know, it didn't say anything. There was nothing about, well, about genetic testing or anything in the paper."* (# 9) *"Yes, but that the ophthalmologist says that there is no eye effect now, but that they cannot rule out that it will come later and that they want to follow up and the ear doctor says that it is 100% not an Usher diagnosis. Who to trust? Who should we listen to? And we think that the ophthalmologist can answer what is about the eyes and the ear doctor can answer what is about the ears."* (# 6)
	Insufficient information	*"It was like a piece of paper at home, then it was no more than that. You might have wanted to ask it straight away and not think about it. Even if you don't get any answers, it still feels more comforting to be able to talk to someone about it."* (# 1) *"We didn't get a lot of information really, in general, neither about syndromes nor this genetic test, but the only thing we really got was that you do a genetic test to rule out that you have a syndrome. That's what we got really"* (# 9)
	No reassurance without genetic information	A parent who wanted to take a genetic test, but the doctor hesitated and delayed the genetic testing. *"It was like this... you don't have to worry, all the time."* (# 2) *"...and then Usher was the first thing that came up and I know I said to the doctor at the time when we found out he was deaf that 'he doesn't have Usher syndrome?' I said. 'No, no, no, God no' she said. 'We shouldn't believe that, absolutely not, we can't imagine that' and so he had it then. There's very little chance of getting Usher, it's a very small percentage who get it. So, of course, it's not very common. But the risk of me and my husband carrying the same, this, is quite unlikely. But it was tough, it was. It was very tough, actually."* (# 9)
Inconclusive test results were stressful	Test results is not reliable	*"Whatever it is, if someone tells you that it's not one hundred per cent reliable, you still rely on it. Well, okay, he's got nothing, so we can rest easy, and if it turns out later that there is something, then that's where the risk lies."* (# 9)
	Vague test results cause concerns	*It's these grey zone cases, that's it, it's hard not to get a clear answer."* (# 6)
Uncertainty if genetic testing is in the best interest of the child	Child and genetics do not harmonies	*"And then if you already know the genetic information about someone like that. Because it doesn't say much, it says he's deaf, but it doesn't say he has two functioning cochlear implants and sign language, signs with support and..."* (# 8)
	The test is not used for the wellness of the child	*"That it falls into the wrong hands. That's the risk, and I feel that the world is not so risk-free anymore. That you should not be completely naive. That's what it is."* (# 4)
	The child´s opinion about genetic testing is unknown	*" That is if you believe that he, we chose to find out everything about him and his genetics. Right now, as his parents. But maybe he doesn't want to know why. Maybe he just accepts that this is the way it is and doesn't want to know more.* (# 8)

Table 6. Quotes and basic and organizing themes in the second global theme

GLOBAL THEME 2 – GENETIC KNOWLEDGE IS CONSIDERED IMPORTANT FOR THE FAMILY AND THE FUTURE		
Organizing themes	**Basic themes**	**Quotes (study participant)**
Easy to decide to do the test	An ordinary test	*"For us it was more, leave sample, blood test. Stick in the arm and they are done, then we wait for a letter in the post. There was not much more."* (# 7)
	Considered important	*"We wanted to know what the cause was, and it was the least we could do, to leave some samples and see what the result is. So it was not a difficult decision. I think we decided already during the first meeting with the doctor, where we were asked the question."* (# 7) *"Yes, but we felt that we wanted to have a chance to find out as much as possible. Especially if there would be any more co-morbidity, or something with the heart or the kidneys or something that we should keep an eye on in the future, so that we can get help with that."* (# 8)
	Altruistic: for the future, the research, for others	*"The main reason we said yes is that we simply wanted to contribute to the fact that, if there is research into this, we want to be on board because, well, if you can come up with something and even if it can't help us or our children, or... maybe it can help someone else who suffers the same in the future."* (# 3) *"It is good to know for other children, for other parents"* (# 5)
Knowledge makes the future predictable	Comprehensive genetic information for ENT specialist	*"It was discussed, yes, it was a good answer, it was a good conversation."* (# 4) *"We have received the information we needed. Why it has happened and so on."* (# 5)
	Satisfaction with verbal and written info from geneticist	*"We went through, in detail, like what it's about, what symptoms you can get and what it looks like in the inner ear and etcetera, etcetera. So we got great information. We have all the information."* (# 7)
	Relief about both what was found and what was not found	*"We were a bit worried about the Usher syndrome, with deaf blindness. It didn't show anything, I guess they would have found that on these tests then. Then they found nothing. That was reassuring."*(#1) *"It was really just a relief that had been released from the shoulders that, well, that you found out how it had happened. Instead of just walking there in your mind without knowing why it has happened."* (#5)
	Knowledge a benefit in itself	*"We chose to do this because we wanted to know"* (# 6) *"I want to know, even if I get a very sad message, I still want to know"* (# 9)
	Facilitates understanding	*"If we had still been walking around suspecting that there was something wrong with the birth, we might have felt worse, or very bad about it."* (# 7) This was also an issue recognized and mentioned by a mother with a negative genetic test. *"It would have felt good to be able to tell my child. Explain to her why she has it, so that she had answers. It would have been easier to understand it"* (# 3)
	Preparation and control	*"We think it's comforting to know like what we can expect, what problems X might have in the future, what can we help him with" (# 8)* *"It gives us the chance to prepare him and that he always has it somewhere in the back of his mind at all times"* (# 9)
	…but makes no difference	*"It doesn't really change anything. You love your child anyway, it's more because you want to be prepared"* (# 2)

Practical use of genetic information in contact with others	Medical assessment affect habilitation and social planning	*"Yes, but of course we wanted to know that. It's just that when it comes to an eye disability too, like blindness, then you also have the world's chance to give him opportunities from the beginning instead of finding out when he's 10 years old. Then just change life and everything. I mean just such a simple thing like we live in Örebro county; we have Sweden's largest deaf and blind school. Imagine if we had planned to move away from here and then we find out when he is ten years old that he is starting to go blind." (# 9)* "*If you know that she will have a change in her hearing over time, or become deaf, for example. Then you can learn sign language."* (# 2)
	Education of family and friends	"*Now I am talking about close family and friends of ours who, when we told them that our daughter has hearing loss, many of them started floating away in their thoughts and reading on the internet without knowing anything. But getting this answer has helped us a lot and put some stop to the speculation going on around us. Because it hasn't been easy to hear others speculate about our child."* (# 7)
	In contact with health care institutions	*"In the emergency room he sees an ear specialist, but that was not what we were looking for. We wanted help and advice if we could give him fluid replacement, what would we give ... but it automatically becomes that... so now we've learned a little bit more that, now we can say that, yes, he has Waardenburg, and this is not due to that."* (# 8)
	In contact with insurance companies	*"Then we can say that there are no more expected diseases or so that are due to this. His hearing loss is sort of self-inflicted and his poor vision is because he is nearsighted, not because of his…* [Waardenburg]*"* (# 8)
	For sibling assessment and family planning	*"For our family, I think it was this, what can I say... checking up on the siblings."* (# 6) *"Because it affected, well, whether you would have more children or not. So that also became a thing. We also got to be part of the result. Even though we originally did it for X's sake, we also got something out of it."* (# 8)

Table 7. Quotes and basic and organizing themes in the third global theme

GLOBAL THEME 3 – KNOWLEDGE ADDS COMPLEXITY AND CAN BE CHALLENGING		
Organizing themes	**Basic themes**	**Quotes (study participant)**
Knowledge can cause worries and influence decisions	Unexpected result cause worries	*"It is terrible. It really was. It was very, very difficult, so... Just Ushern then. Because I had read a lot, just when he was born deaf. So, I read a lot about what different syndromes it could be related to. That's what you do, you look it up."* (# 9) There is also a risk of worrying in advance, afraid of unexpected results that never happens. *"I can imagine that there may be things that you might not want to know if it is not possible to do anything about it. If you have a greater risk of getting certain diseases or so, it may not always be fun to know. However, it's good if you can do something about it and detect it early. But that's the risk you take, to worry unnecessarily."* (# 3)
	Can influence decision on more children	*"We learnt that it was recessive, and all our children have a 25% risk of getting both mutations. Then we felt that we shouldn't have any more children and if I had become pregnant, we would have chosen to have an abortion.* (# 6)
Human suffering seen as a reason for prenatal testing and abortion	Syndromic SNHL reason for abortion	*"Let's take that as an example, if I had known that, and say she had Usher syndrome, I would not have had her. I can say that one hundred per cent."* (# 1) *"I think it's more difficult with syndromes and things like that, where maybe you could... it doesn't just have to be for your own sake, but it can also be for the sake of the child, that this might not be the life you want for someone, so to speak."* (# 2) *"Then when it comes to me and the father who carries this and has a 25 percent risk of having a child with Usher, then of course you wish you had been tested earlier. So on a societal level, I mean, because it's a burden, even though X is our child, we love him more than anything, but it's still a burden on both the healthcare system and his future as well, yeah, how to explain this in a nice way."* (# 9)
	Isolated SNHL is not regarded as a reason for abortion	*"X's hearing loss is not that severe. She is doing well anyway. So, it wouldn't have been a decisive factor if fetal diagnostics had been possible."* (# 3) *"I don't think we would have rejected a child if we had been told it was just an isolated hearing loss. We would never have done that."* (# 6)
Thoughts about selection and normality		*"It is not something that affects anyone else that I find out if my child has a syndrome."* (# 2) *"But this is a big issue with ethics, because it involves selecting what is normal and what is not normal. And that is very difficult. Very, very difficult."* (# 6) *"And to opt out in any way. No. They have a great life, and they are great boys. So based on what has been when they were born and what is now, that's it. You can't opt out of that."* (# 4)

Results

The findings in this study are based on nine interviews of ten parents of children with SNHL, examined with WGS. From the transcribed interview data, three global themes were identified. 1) Limited knowledge creates uncertainty 2) Genetic knowledge is considered important for the family and the future, and 3) Knowledge adds complexity and can be challenging. Each of the global themes has three organizing themes based on basic themes presented in **Figure 18**.

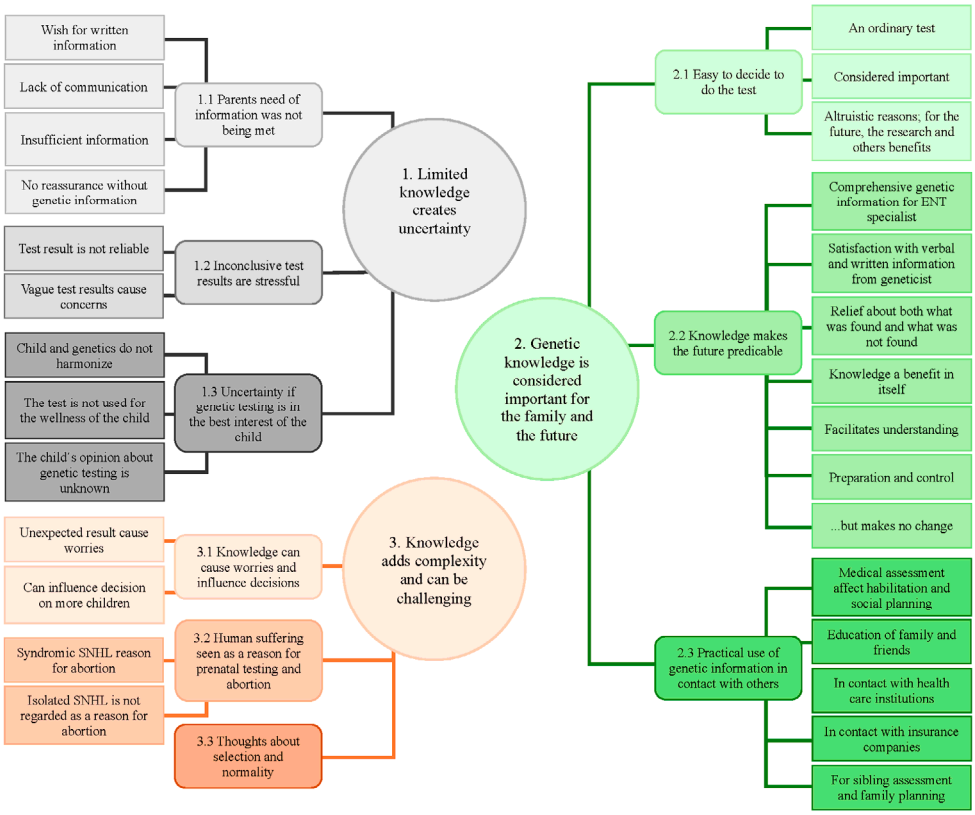


Figure 18. Thematic network of the global themes, organising themes, and basic themes.

The second theme dominated. The parents had different perspectives on knowledge, from reasons to why they wanted to have the genetic test done, also altruistic reasons, to practical use in contact with others. Furthermore, the knowledge was utilized to make the future predictabel, which was based on experiences like relief about the result, knowledge as beneficial, and as a facilitator for understanding.

The first and third global themes provide important information that clinicians should be aware of when performing genetic testing on children with SNHL. In the first global theme, "Limited knowledge creates uncertainty", parents expressed that their need for information was not being met. They also felt that the test results themselves could be stressful and unreliable if no definitive genetic diagnosis could be made. In the final global theme, knowledge could influence more complex decisions. Among other things, parents expressed that syndromic HL could be a reason for abortion, while isolated HL was not perceived as such.

The two researchers agreed on the coding and thematization. The consistency in the co-judging, known as inter-rater reliability, between the two researchers increased

credibility. Repeatedly reviewing the themes and quotes also ensured alignment between the themes and the experiences that were actually expressed verbally. There was consistency in the research process, with the same researcher conducting all interviews, transcribing, coding, and analyzing, as well as taking notes related to the interview situation. Dependability was achieved through the consistent and transparent process. Transferability was assumed based on the similarity to a previous study conducted in an Australian context[116]. To ensure confirmability, triangulation was used with several co-authors, who also analyzed themes and assessed the plausibility of the analysis results.

Discussion

The conclusion is that parents experienced genetic testing as personally valuable and practically useful, even though there are no treatment options available. Conversely, ambiguous or vague results can cause significant difficulties.

Analysis of uncertainty has found that it is linked to anxiety[134,135]. Furthermore, studies have shown that uncertain results can cause anxiety levels comparable to those associated with known negative consequences[136]. Managing uncertainty involves providing available information and knowledge, while also acknowledging existing gaps in knowledge. Based on the parental request for information, it is likely beneficial to be informed about the uncertainty associated with genetic testing. The information should relate to the current level of knowledge in order to best handle the situation.

When it comes to knowledge, the discussion aligns with Antonovsky's sense of coherence theory, which connects comprehensibility to both manageability and meaningfulness[137]. Tutty et al.[116] described how the genetic test result provided a sense of control and empowerment, which correlates well with our results. Therefore, it is reasonable to believe that our findings are tranferable to other settings.

In addition, parents appeared to be able to identify, make informed decisions, and take a stance on complex ethical issues.

This study of parental experiences reveals that genetic testing for children with HL is predominantly beneficial for their families. However, it is essential that parents are well-informed and aware that only about half of cases result in a genetic diagnosis and that even a genetic diagnosis sometimes does not provide a complete picture. Therefore, if the doctor suspects a genetic cause for HL and the parents are eager to find an answer, there is no need to hesitate to perform a genetic test.

Concluding discussion of the thesis

Genetic diagnostics and WES/WGS are highly relevant topics in both medicine and society, extending beyond the scope of audiology and HL diagnostics. Genetic testing is applied across multiple disciplines and for various purposes, including ancestry determination in commercial laboratories, cancer diagnostics, and pre-implantation genetic diagnosis (PGD) for identifying known parental pathological traits.

The complexity of genetic testing is illustrated in this thesis by including various topics related to genetic HL. This thesis covers a wide variety of topics including knowledge about HL related genes and inheritance patterns to parental experiences of genetic testing, including psychological reactions and practical consequences. Other aspects to consider regarding genetic tests, which are not addressed in this thesis, include ethical and socioeconomic factors.

Clinical decision-making should be based on established medical evidence, including knowledge from biochemical and genetic disciplines. Furthermore, understanding patient perspectives on the diagnostic process, results, and treatment is essential for delivering person-centered care. To make the thesis clinically useful, I aimed to provide the basis for guidelines on who should undergo genetic testing. However, this task proved to be delicate, and the reasons will be further discussed.

In children with moderate to severe HL, there is currently approximately a fifty percent probability of identifying a genetic background. In our cohort (Studies I and II), the diagnostic yield was 43% with findings in 25 different genes. According to the literature, the diagnostic yield is decreased when patients with conductive HL, mild SNHL[138], adults[26], or patients with unilateral HL[139,140] are tested. However, focusing solely on genetic yield may not be decisive when choosing whether to perform a genetic test. There are other diagnoses for which a lower diagnostic yield is considered relevant. In the Danish guidelines for genetic testing of patients with HL, the possibility of testing adults, unilateral HL, and mild HL is included[141]. In a study from Denmark on 100 CI-treated patients, whereof 20 patients had single-sided deafness (profound unilateral HL), they found a genetic cause in 44 cases (44%), and three of those had single-sided deafness[142]. In Sweden, the attitude toward genetic testing has been somewhat more restrictive. This is probably based not only on the expected diagnostic yield, but also on the estimated benefits, current cost of testing and clinical traditions.

Whom to test?

The question remains whether everyone with HL should be offered testing or whether it is more reasonable to prioritize certain groups. It seems reasonable to prioritize children with moderate to profound HL for testing. However, among children with mild HL, there are some who suffer from progressive HL. This group could benefit from a conclusive genetic test result for prognostic purposes. Therefore, to draw a specific line in the hearing threshold according to 4fPTA is not that simple. The importance of knowledge for parents of children with HL was evident in the interview study (Study IV) in this thesis. This knowledge is likely related not only to the genetic test itself, but also to reasonable expectations of the test. Informed parents, who have children with unilateral and mild HL, who know that the likelihood of finding a genetic cause is limited, are probably likely to refrain from doing genetic testing. Based on this reasoning, genetic sequencing can be discussed with all parents and offered to all children with HL, but without the intention to actually test all of them.

Among adults with a later onset of HL who have undergone genetic testing, autosomal dominant traits are more common[26]. In light of this, it can be argued that young adults and middle-aged individuals of childbearing age with onset or progression of HL should be offered genetic evaluation. This is based on the fact that the risk of passing the disease on to their children increases with dominant inheritance patterns. This reasoning is complicated by the fact that HL in adults is often multifactorial.

Non-participants

A limitation of this thesis is that children in families who have decided not to do genetic testing on their children are not studied. Identifying any shared characteristics of this group would be interesting. It would be interesting to understand whether parents' country of birth, level of education, religion, political views, or other background factors influence the decision to participate in a genetic study. Hypothetically, language, educational level, and cultural aspects can constitute barriers to genetic testing. Experiences, both personal and societal, as well as the ability to understand the benefits and/or risks associated with genetic testing, can likely influence decision-making regarding genetic testing. Parents may also believe that decisions related to the genetics of the child should be deferred until the child can make informed choices.

The results in Study IV are based on parents who decided to have their child undergo genetic testing and who were able to communicate in Swedish. Accordingly, here is therefore also a risk of bias in the results, both in terms of attitudes to genetic testing and the influence of cultural background.

Genetic screening, genotype prior to phenotype

The clinical utility of genetic testing is well established in different clinical settings, underscoring its relevance. For a correct genetic diagnosis, the genotype should correspond to the phenotype. In modern medicine, the phenotype is often identified first, unless it is a known hereditary disease within a family. In the future, the diagnostic process might be reversed, and the genotype might be used to identify the phenotype instead. A step in this direction is the implementation of genetic sequencing as a population screening tool. For example, Genomics England, together with the National Health Service (NHS) has started a "Newborn Genomes programme"[143] with the intention to sequence 100.000 newborns for around 200 rare diseases. When choosing the diseases, they adhered to the principles that the evidence for pathogenicity should be strong, the penetrance should be high, presymptomatic interventions should be desired, treatment should be available, and cost-effective for society. There are similarities with the screening criteria Wilson et al. presented in 1968, but there is an ongoing debate on how to adapt screening programs in the genomic era[144,145].

There are similar projects with genomic sequencing screening programs for newborns around the world[146], among others, the GUARDIAN study in New York, USA[147,148], the Baby Screen+ in Melbourne, Australia[149] and the BabyDetect study in Belgium[150]. Although there are high expectations for genetic screening worldwide, there are also skeptical voices regarding this development, who are more inclined to advocate a targeted approach based on symptomatology[151].

In Heidelberg, Germany, an ongoing project called NEW_LIVES exists, which is a genomic newborn screening program (https://www.klinikum.uni-heidelberg.de/en/new-lives-genomic-newborn-screening-programs). This program also consider ethical, social, and legal issues before introducing genomic screening. In a qualitative study with a focus group, they stated that "Identifying uncertainties and addressing them in implementation and education is crucial." This raises important questions regarding appropriate boundaries for use of genetic sequencing, which can be related to genetic testing in general, as well as WGS for SNHL. That uncertainties and limited knowledge pose problems when doing genetic testing was also one of the global themes in our qualitative Study IV.

Since hearing screening with OAE tests has a high sensitivity, genetic hearing screening, which has a much lower sensitivity, is questionable. Even if it hypothetically had been practically feasible. At present, however, this must be considered too costly in terms of both labour and resources.

In Ontario, USA, pathogenic variants in *GJB2* and *SLC26A4* (Pendred syndrome) were included in the newborn hearing screening, using the blood-patch test. The pathogenic variants were detected with MassArray and Sanger sequencing. In the study, they found that an early genetic diagnosis led to an earlier diagnosis of

profound SNHL compared to traditional hearing screening with OAE, and therefore, an earlier treatment with cochlear implants[152].

Based on the heterogeneous genetic background of HL, screening for PVs (22 PVs in *GJB2* and *SLC26A4*) in two genes appears to miss too many of the relevant variants in genes associated with SNHL. Among the PVs identified in our studies, only six (n=6/96) patients would have been identified using the variants they included in their test[152]. Note that this is in a cohort of patients with HL, not as a screening tool in the population. A screening tool should be sensitive for finding HL in the population. However, this screening technique must be considered unreasonably sensitive to find children with HL, even if the technique is valid and reliable to identify the specific PVs.

Another approach could be to consider screening for specific syndromes, where the burden of comorbidity is particularly high or where there is a risk of mortality related to the genetic variant (e.g., Jervell-Lange Nielsen). Usher syndrome is one of the most common syndromes related to HL, and our interview study shows that it is the syndrome that parents are most concerned about. However, in Usher syndrome, the heterogeneity of the genes involved is also problematic, with varying penetrance, as well as the lack of treatment for vision loss.

Testing for mt1555A>G and mt1494C>T has also been proposed to avoid aminoglycoside-induced non-syndromic SNHL. With a rapid genotype test, neonates (751 children) were sceened when admitted to intensive care units[153] to avoid aminoglycoside-induced SNHL. Three neonates with the m1555A>G variant were identified. In these cases, aminoglycosides were avoided without delay of antibiotic administration. A successful screening to avoid SNHL in a targeted group.

In conclusion, I do not support genetic sequencing in newborn screening for HL with a sensitivity of WGS of around 50%. Instead, I believe that targeted analyses with gene panels for children with confirmed HNS are preferable. With the expanding genetic knowledge and future possibilities of utilizing artificial intelligence to upgrade variants of uncertain significance, this standpoint may be revised in the future. Genetic treatment can also be a game-changer in the views on genetic newborn hearing screening. If the aim for screening was to find treatable PVs causing HL, the criteria for screening would be applied differently.

Future perspective on genetic SNHL

There is an ongoing scientific revolution regarding genetic therapy for HL. Deafness can be cured. Not all deafness, but if it happens to be caused by a PV in *OTOF,* it can be cured[96,97]. Numerous studies, the majority on mouse models, but some on larger animals including primates, have been conducted prior to the study on humans[91,98,154-158] However, long-term data remains unavailable at the time of writing. Furthermore, whether genetic therapy offers advantages in hearing function compared to cochlear implants has not yet been established.

The heterogeneity poses a problem for genetic therapy in SNHL, in congruence with the genetic testing and screening discussed previously. In our cohort, only one of 96 patients exhibited a PV in *OTOF*[138,159]. Thus, there are numerous additional genes that require investigation in relation to gene therapy. The pathomechanisms and morphological prerequisites associated with each gene need to be understood. Critical factors for gene therapy are that the morphological structures of the cochlea need to be intact, or that genetic treatment need to occur before the development of the abnormal structures of the inner ear[160]. The inner ear is fully developed in humans at birth, unlike in mice, where the inner ear continues to mature after birth. Experiences from experiments on newborn mice may therefore be difficult to transfer to humans.

Advances and complexity of genetic treatment

As expected, *GJB2* was one of the most prevalent genes with PVs in our population[27]. Finding genetic treatments for *GJB2*-variants is, of course, appealing. However, it has been challenging to find an effective treatment strategy. This is partly due to genetic diversity, with more than 300 different PVs described in *GJB2*[161]. Also, for gene replacement to be successful, the gene sequence must reach the affected cell types. The protein, Connexin 26 is expressed in various cochlear cell types, including supporting cells, stria vascularis, and spiral ligament, which use Connexin 26 for their gap junctions, enabling, among other things, the transfer of potassium. Another important issue that poses problems with gene therapy related to *GJB2* PVs is that ectopic Connexin 26, i.e., if the protein is expressed in the wrong place, is ototoxic[161].

Different virus vectors can be used to deliver gene therapy into the cells. The adeno-associated virus (AAV) vector is commonly used due to its safety and efficacy[162,163]. The cargo capacity of AVV vector is limited to 4.7kb, which is problematic when delivering larger sequences. Techniques involving duplication of AAV (duo-AAV) and overloading single AAV strategies[98] have been used.

It has been challenging to find an AAV vector to target the cell types expressing Connexin 26. However, in a recent study[164], these challenges were addressed by testing different serotypes of AAV vectors to enhance the specificity and efficacy of the injected therapy. Additionally, they explored various promoters for targeting cells expressing Connexin 26, achieving promising results in restoring hearing in *Gjb2*-deficient mice.

The challenge of developing genetic treatment depends on the genetic variant and in which cells the related protein is expressed and acts. If a PV affects the development of morphological structures during the embryonic period, as the lack of pendrin does in Pendred syndrome (*SLC26A4*)[165], this adds another level of complexity to genetic treatment[162].

Different approaches to restoring hearing/gene therapy

Gene therapy can use different strategies depending on the pathological background, and various vectors and approaches for delivering the treatment to the inner ear have been tested[160,162,163].

Gene replacement strategies are suitable for HL with an AR inheritance pattern, as shown in the successful *OTOF* study[92], where functional gene sequences were added. In AD inheritance patterns, an interfering RNA molecule can cause gene suppression of the dominant allele, as demonstrated in a study on *TMC1*-related deafness[166]. In this approach, the dominant, but non-functional sequence is shut down. Gene editing techniques, such as CRISPR/Cas9 and base editing, which correct the existing sequence in the genome, are also potentially viable strategies[167]. Another potential strategy is to generate inner ear organoids from human pluripotent stem cells (iPSCs)[168,169]. Thus, there are many possible strategies, and the future will show which of these will be useful.

In summary, genes that express a protein in a specific cell type with intact morphology, and an effective mechanism for regulating genetic expression should be the focus of successful gene therapy.

Populärvetenskaplig sammanfattning på svenska

Hörselnedsättning/dövhet är den vanligaste medfödda sensoriska nedsättningen och drabbar en till två av tusen nyfödda. I de flesta fall finns en genetisk förklaring till hörselnedsättning, som kan vara antingen isolerad eller del av ett syndrom.

Den genetiska bakgrunden är heterogen, det vill säga det finns patogena varianter i många olika gener och det finns flera hundra beskrivna gener som är relaterade till hörselnedsättning, både isolerad och syndromal. Det vanligaste nedärvningsmöntret är autosomalt recessivt, dvs att det krävs både ett förändrat arvsanlag från mamman och ett från pappan för att det ska resultera i hörselnedsättning. Autosomalt dominant nedärvningsmönster, där det endast behövs en patogen variant för att få symtom förekommer i ungefär tjugo procent av fallen. Könsbundet nedärvningsmönster, som är knutet till könskromosomen, och maternellt (via mitokondrierna) nedärvningsmönster förekommer också, men endast i mycket begränsad utsträckning. Mitokondrierna är förutom cellkärnan de enda organellerna med eget arvsanlag eller DNA. Mitokondrierena ärvs från modern då de följer med äggcellen. Många av proteinerna som behövs i mitokondrierna kodas av cellkärnans DNA, medan en del av proteinerna kodas av det mitokondriella DNA:t. Mitokondriesjukdom kan därför bero både på genetisk variation i kärn- och i mitochondrie-DNA.

Genetisk sekvensering är en kraftfull metod för genetisk diagnostik. Sekvensering av den proteinkodande delen av arvsanlaget (helexomsekvensering) eller hela arvsanlaget (helgenomsekvensering) kan utföras utifrån DNA som extraherats från till exempel celler i blodet Vid hörselnedsättning analyseras ofta den genetiska informationen utifrån en genlista, en så kallad genpanel, vilket innebär att man avgränsar avläsningen till utvalda gener. I våra studier har vi använt en genpanel (HearSeq) som kureras kontinuerligt och innehåller runt 200 gener.

Målet med denna avhandling var att studera den genetiska variationen i vår population relaterat till hörselnedsättning. Dessutom ville vi undersöka vilken erfarenhet föräldrar till barn som testats genetiskt hade av den genetiska testningen. För att förstå hur mitokondriellt relaterad hörselnedsättning uttrycks gjordes även studie på patienter som diagnostiserats med mitokondriell sjukdom.

Genetisk diagnostik med sekvensering av arvsmassan utfördes på barn och även en del vuxna med lindrig till grav hörselnedsättning, som diagnostiserats vid de audiologiska mottagningarna vid universitetssjukhusen i Lund eller i Örebro åren 2019–2022. Alla medverkande, eller deras föräldrar, har fått muntlig och skriftlig information och skriftligt godkänt medverkan i studien.

Tio föräldrar, till barn med hörselnedsättning som testats genetiskt, har djupintervjuats. Intervjuerna har sedan analyserats kvalitativt med tematisk analys metod. Detta är en metod som innebär att innehållet kodas och tematiskt stuktureras utifrån det empiriska innehållet.

Medicinska journaler för alla patienter som diagnostiserats med mitokondriell sjukdom (n=197) vid barnsjukhuset i Philadelphia under åren 2008 till 2019 granskades angående hörsel- och genetikdata. Patienterna grupperades utifrån underliggande genetisk patologi, uppdelat i mitokondriesjukdom orsakat av patogena varianter i kärn-DNA, varianter i mitokondrie-DNA och större deletioner (när del av den genetiska koden saknas) av mitokondrie-DNA. Det visade sig att mer än en fjärdedel av patienterna med mitokondriell sjukdom hade verifierad hörselnedsättning. Hörselnedsättning var vanligast i gruppen med mitokondriedeletioner. I alla fall där mitokondrie-DNA var påverkat (antingen som en patogen variant eller som en deletion) debuterade hörselnedsättningen i skolålder, tonår eller ung vuxen ålder.

I de två prospektiva studierna där genetisk variation studerats med hjälp av helexomsekvensering (n=11) och helgenomsekvensering (n=85) kunde en genetisk diagnos konstateras i 43% av fallen. Hos de med lindig hörselnedsättning, vilket endast var tolv stycken, kunde en genetisk diagnos endast kontateras i ett fall. Vid hörselnedsättning som var medfödd eller som debuterat innan 2 års ålder konstaterades en genetisk orsak i mer än hälften av fallen. Mer än en tredjedel av patienterna hade föräldrar med ursprung i mellanöstern och i denna grupp konstaterades autosomalt recessivt homozygot nedsärvningsmönster (identiska genetiska varianter från båda föräldrarna) i nästan hälften av fallen. I gruppen med föräldrar födda i Sverige hade endast en av tio homozygot nedärvningsmönster. Patogena varianter konstaterades hos 39 patienter i 25 olika gener. Hos 15 patienter konstaterades att hörselnedsättning var del av ett syndrom, där Usher syndrom (n=8) och Pendred syndrom (n=3) förekom i störst utsträckning.

Vid intervjustudien identifierades tre teman, som alla var relaterade till den genetiska kunskapen. Första temat handlade om hur begränsad information och tvetydighet i det genetiska utfallet skapade osäkerhet och i förlängningen oro och ångest. Andra temat, med flest underteman, handlade om hur viljan att få mer kunskap var självklar för föräldrarna. De upplevde också att den genetiska kunskapen hjälpte dem att förstå, förklara och hantera situationen och omvärlden. Dessutom var den genetiska kunskapen till praktisk nytta i relation med andra, i kontakt vården och andra organisationer. Tredje temat handlade om att kunskap kan

leda till komplexa etiska funderingar och ställningstagande, samt kan influera familjeplanering.

Genetisk sekvensering är en effektiv metod för att identifera orsaken till hörselnedsättning. Hos barn med tidig debut av måttlig till grav hörselnedsättning kan en genetisk orsak identifieras i mer än hälften av fallen. Mitokondriell sjukdom bör beaktas vid debut av hörselnedsättning från skolålder till ung vuxen ålder och framför allt vid symtom från andra energikrävande organsystem. Föräldrars upplevelse av den genetiska testningen är övervägande positiv och hjälper till att göra framtiden förutsägbar och har praktiska implikationer. Genetisk diagnostik är ett fält som expanderar och på sikt kommer genetisk behandling att utvecklas. Avgörande för om genetisk terapi är möjligt kommer vara beroende av vilken gen som påverkats och dess verkningsmekanismer.

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Artificial intelligence declaration

The entire manuscript was written by the author. Grammarly was used for spelling and grammar correction. DeepL was used to translate quotes from Swedish to English in the interview study. The translations were corrected manually and retranslated. Scopus AI was used to search for articles alongside traditional article searches in PubMed.

Supplements

Supplement Table 1. The Swedish original questionnaire developed for study II

	Uppföljning efter studien av genetiska orsaker till hörselnedsättning **Ringa in de svar som stämmer överens med din upplevelse** **Barnets ålder vid medverkan i studien:_____**			
1	**Jag tillfrågades och fick information om att vara med i en studie om genetisk utredning av hörselnedsättning:**			
	Stämmer mycket bra	Stämmer bra	Stämmer dåligt	Stämmer inte alls
2	**Utredningen konstaterade att hörselnedsättningen var genetiskt orsakad:**			
	Ja	Nej	*Om du ringat in **NEJ** kan du fortsätta direkt till fråga 19*	
UTREDNING/INFORMATION				
3	**Jag fick ökad kunskap om det ärftliga tillståndet:**			
	Stämmer mycket bra	Stämmer bra	Stämmer dåligt	Stämmer inte alls
4	**Jag förstår hur kunskap/information om det ärftliga tillståendet kan förmedlas till mina släktingar:**			
	Stämmer mycket bra	Stämmer bra	Stämmer dåligt	Stämmer inte alls
5	**Jag erhöll information:** **(Markera de/det alternativ som stämmer bäst för dig)**			
	Vid mottagningsbesök	Skriftligt i brev eller e-post	Telefonsamtal	Informationsbroschyr
	Annat sätt	Jag fick ingen information (om ja, gå till fråga 9)		
6	**Den muntliga informationen var tydlig:**			
	Stämmer mycket bra	Stämmer bra	Stämmer dåligt	Stämmer inte alls
7	**Den skriftliga informationen var tydlig:**			
	Stämmer mycket bra	Stämmer bra	Stämmer dåligt	Stämmer inte alls
8	**Jag hade önskat erhålla information på detta sätt:**			

UPPFÖLJNING				
9	**Jag fick veta vilken uppföljning som är viktig för mitt barn:**			
	Stämmer mycket bra	Stämmer bra	Stämmer dåligt	Stämmer inte alls
10	**Jag har förtroende för den medicinska bedömningen som gjorts:**			
	Stämmer mycket bra	Stämmer bra	Stämmer dåligt	Stämmer inte alls
TILLGÄNGLIGHET				
11	**Det var lätt att komma i kontakt med mottagningen:**			
	Stämmer mycket bra	Stämmer bra	Stämmer dåligt	Stämmer inte alls
12	**Det togs hänsyn till mina önskemål gällande tid till mottagningsbesök:**			
	Stämmer mycket bra	Stämmer bra	Stämmer dåligt	Stämmer inte alls
13	**Har du några synpunkter på tillgängligheten under den genetiska utredningen, till exempel kallelser, telefontider, väntetider, resvägar eller annat?**			
BEMÖTANDE OCH DELAKTIGHET				
14	**Jag möttes med respekt:**			
	Stämmer mycket bra	Stämmer bra	Stämmer dåligt	Stämmer inte alls
15	**De jag mötte eller hade kontakt med förstod min situation:**			
	Stämmer mycket bra	Stämmer bra	Stämmer dåligt	Stämmer inte alls
16	**Jag förstår att information om mig, min familj och släkt är viktig för den genetiska utredningen:**			
	Stämmer mycket bra	Stämmer bra	Stämmer dåligt	Stämmer inte alls
17	**Mina erfarenheter och kunskaper om den ärftliga sjukdomen efterfrågades:**			
	Stämmer mycket bra	Stämmer bra	Stämmer dåligt	Stämmer inte alls
18	**Mina frågor besvarades:**			
	Stämmer mycket bra	Stämmer bra	Stämmer dåligt	Stämmer inte alls
19	**Jag upplever att den genetiska utredningen gav ett mervärde för mig och min familj:**			
	Stämmer mycket bra	Stämmer bra	Stämmer dåligt	Stämmer inte alls
20	**Jag skulle rekommendera andra familjer med barn med hörselnedsättning att genomgå denna typ av utredning:**			
	Stämmer mycket bra	Stämmer bra	Stämmer dåligt	Stämmer inte alls
21	**Har du andra tankar/funderingar/reflektioner angående den genetiska utredningen?**			

Enkäten baserad på, men med enstaka modifieringar och tillägg, en enkät som använts vid studien ”UTVÄRDERING AV PATIENTUPPLEVD KVALITÉ OCH TILLFREDSTÄLLELSE VID KLINISK GENETISK MOTTAGNINGSVERKSAMHET” av Karin Svensson, Genetisk rådgivare, Klinisk Genetik, Lund, SUS. Tillåtelse till att använda enkäten har hämtats av upphovsmannen.

Supplement Table 2. Semistructured interview guide study IV

Semi structured interview guide

HearSeq2: Qualitative descriptive interview study of how parents of children with moderate to severe hearing loss experience genetic testing with whole genome sequencing (WGS)

Thank you for taking part in this study!

Verbal introduction

This is, as you probably already understood, an interview study. The interview is estimated to take between 45 minutes and one hour. We are doing this study to understand how parents of children with hearing loss who undergo genetic testing perceive the test and the genetic test result. Furthermore, we like to examine whether there are any perceived benefits or risks of the test. The interview will be recorded and analyzed and stored in our research department. If you have not already done so, you will be able to read the information before we start. If you want to participate, I need your written consent.

1. **The genetic test result -present tense**
    - Do you know if your child has a genetic diagnosis?
    - Can you tell me about what this diagnosis means?
    - How do you feel about having a child with this diagnosis? Alternatively, how do you feel about your child not having a confirmed genetic diagnosis?
        - What are your thoughts/feelings about this?

2. **Information – past tense**
    - When did you find out that your child has a hearing loss
    - When was the genetic testing done?
    - How did you get the information from the genetic testing?
    - How did you perceive that information to be?
        - Why did you feel that way?
        - Can you give examples?
        - Would you like the information to be given in any other way?
    - What benefit did you feel you got from the information when you received it?
        - In what way?
        - why not?
    - Do you know if the answer to the genetic test led to further action?

3. **Follow-up – past tense**
    - Did you find out why your child has a hearing loss?
    - How was it experienced?

4. **Expectations – past in relation to present tense**
    - What were your expectations of what genetic testing would mean for your child?
    - What were your thoughts before the test?
    - How did you react to the results of the test?
    - Were your expectations in line with what happened?

5. **Emotions related to the genetic testing - past and present**
    - Did you have any emotional reaction to the result of the genetic testing?
    - What are your feelings today about your child having a disability that may be hereditary?

6. **Benefits and risks – present**
    - Do you feel that genetic testing is beneficial for you?
    - Do you feel that there are risks associated with genetic testing?
    - Can you give examples?
    - Elaborate on your reasoning and how you think

7. **Ethics – present**
    - Do you perceive any the ethical aspects of genetic testing?
    - Did it influence your decision to participate in the study?
    - If you wish to have another child, will you/will you use the information obtained from the genetic testing?

8. **Expectations for the future**
    - What do you think genetic testing could mean for your child in the future?
    - What possibilities do you think this technology has in the future?

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Extended genetic diagnostics for children with profound sensorineural hearing loss by implementing massive parallel sequencing. Diagnostic outcome, family experience and clinical implementation

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A B S T R A C T

Objectives: The aim of this study was to investigate genetic outcomes, analyze the family experience, and describe the process of implementing genetic sequencing for children with profound sensorineural hearing loss (SNHL) at a tertial audiological center in southern Sweden.

Design: This is a prospective pilot study including eleven children with profound bilateral SNHL who underwent cochlear implant surgery. Genetic diagnostic investigation was performed with whole exome sequencing (WES) complemented with XON-array to identify copy number variants, using a manually curated gene panel incorporating 179 genes associated with non-syndromic and syndromic SNHL. Mitochondrial DNA (mtDNA) from blood was examined separately. A patient reported experience measures (PREM) questionnaire was used to evaluate parental experience. We also describe here the process of implementing WES in an audiology department.

Results: Six female and five male children (mean 3.4 years, SD 3.5 years), with profound bilateral SNHL were included. Genetic variants of interest were found in six subjects (55%), where three (27%) could be classified as pathogenic or likely pathogenic. Among the six cases, one child was found to have a homozygous pathogenic variant in *MYO7A* and two children had homozygous likely pathogenic variants in *SLC26A4* and *PCDH15,* respectively. One was carrying a compound heterozygote frameshift variant of uncertain significance (VUS) on one allele and in trans, a likely pathogenic deletion on the other allele in *PCDH15*. Two subjects had homozygous VUS in *PCDH15* and *ADGRV1,* respectively. In five of the cases the variants were in genes associated with Usher syndrome. For one of the likely pathogenic variants, the finding was related to Pendred syndrome. No mtDNA variants related to SNHL were found. The PREM questionnaire revealed that the families had difficulty in fully understanding the results of the genetic analysis. However, the parents of all eleven (100%) subjects still recommended that other families with children with SNHL should undergo genetic testing. Specifically addressed referrals for prompt complementary clinical examination and more individualized care were possible, based on the genetic results. Close clinical collaboration between different specialists, including physicians of audiology, audiologists, clinical geneticists, ophthalmologists, pediatricians, otoneurologists, physiotherapists and hearing habilitation teams was initiated during the implementation of the new regime. For all professionals involved, a better knowledge of the diversity of the genetic background of hearing loss was achieved.

Conclusions: Whole exome sequencing and XON-array using a panel of genes associated with SNHL had a high diagnostic yield, added value to the families, and provided guidance for further examinations and habilitation for the child. Great care should be taken to thoroughly inform parents about the genetic test result. Collaborations between departments were intensified and knowledge of hearing genomics was increased among the staff.

1. Introduction

Congenital sensorineural hearing loss (SNHL) is the most common sensory impairment in humans with between one and two newborn children per thousand affected by severe to profound SNHL [1–4], with or without concomitant loss of vestibular function. In high income countries, more than fifty percent of the cases can be attributed to a genetic cause [1,5,6]. SNHL is non-syndromic in seventy percent of the cases [7] and an autosomal recessive inheritance pattern dominates [7,8]. The most common genetic cause is related to variants in the *GJB2* and *GJB6* genes, resulting in disturbed production of connexin protein in the inner ear [8–11]. Non-syndromic SNHL is genetically heterogeneous

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[1,8] and apart from *GJB2* and *GJB6* there is a wide genetic diversity, with known pathogenic variants in at least 124 genes [12]. The number of identified pathogenic variants is steadily increasing due to continuous improvements in sequencing technologies. Variants in genes related to syndromic SNHL, and multi-systemic mitochondrial diseases with SNHL, expand the genetic variation even further.

The shift from single gene or variant analysis to comprehensive diagnosis-specific gene panels represents a paradigm shift in genetic diagnostics over the last decade. Massive parallel sequencing, including whole exome sequencing (WES) complemented with microarrays or whole genome sequencing (WGS), can now be considered gold standard when investigating children with hearing loss [2,3,7,13]. However, a comprehensive genomic diagnostic approach is not yet a standard procedure and is still controversial in some clinical settings. Skepticism may be due to the initial high financial cost or lack of knowledge of what value the improved diagnostic process can bring to the patients. Furthermore, ethical issues of genetic testing need to be considered. This pilot study takes both a family and a clinical perspective on implementation of genetic testing.

In WES the actual protein coding region of the genome, the exome, is sequenced, whereas in WGS the whole genome is sequenced. In three large studies using sequencing technology, a genetic cause of hearing loss was found in 24–40% of the cases [14–16]. Sloan-Heggen et al. (2015) [14] from Iowa, USA, found a genetic cause in 440 of 1119 patients (39%) including structural variants (deletions, duplications, translocations). Nishio et al. (2015) [15] from Japan focused on single nucleotide variants and concluded that 30–40% of the 1120 subjects were deaf due to a genetic alteration. Mehta et al. (2016) [16] from Philadelphia, USA identified the etiology for HL in 24% of 660 subjects, including copy number variants (CNVs). This gene panel also included the well-described *m.1555A > G* mitochondrial mutation. The mtDNA was not examined further in any of these three studies. Several less extensive studies [1,2,13,17–23] have been conducted, identifying a definite genetic diagnosis ranging from 33,5% [1] to 60% [21] of the cases. The difference in diagnostic yield can be understood based on differences in the populations studied, the number of genes included in the gene panels (which varied from 39 [21] to 247 [2]) and whether subjects with previously known variants in *GBJ2/GBJ6* were excluded.

There are challenges for the clinician in making decisions and interpretations associated with sequencing technologies, and basic genetic knowledge is crucial for succeeding with the implementation. First, the human genome is vast and covers about 20,500 protein coding genes [24]. A meaningful output of a genetic analysis requires that genes associated with SNHL are compiled as a list, a gene panel, and that it is regularly updated, preferably by a multidisciplinary team including both audiology and clinical genetics expertise, as new genes are identified as being associated with SNHL. Second, all individuals have variants in their genetic code as each person's DNA differs from the reference genome in millions of locations [25]. Most of these variants represent benign interpersonal variation [5], and the reference genome does not cover worldwide variation. Even in genes with a well-described correlation to SNHL, single nucleotide variants and structural variants do not have to be causal of the symptoms. According to the American College of Medical Genetics and Genomics (ACMG) [26] guidelines, variants are classified as pathogenic, likely pathogenic, of uncertain significance, likely benign, or benign. The criteria are based on combining different types of evidence, graded from strong to supportive toward pathogenicity or a benign state. Variants of uncertain significance (VUS) are normally not reported clinically as they cannot explain the disease at hand with sufficient certainty [27]. Information about phenotypical features and mapping close relatives according to phenotype and genetic variation can sometimes help the geneticist to reclassify the variant from VUS to likely benign or likely pathogenic.

Third, when implementing new genetic test methods, benefit for the child and its family need to be ensured and most importantly we need to address the risk that the testing might be of harm. It has been shown that even in the absence of clinical utility, there can be a personal gain for the patients and the parents to support testing [28]. Hayeems et al. (2016) [29] found in a qualitative study in Canada that genetic testing can bring both an intrinsic, personal gain, and an instrumental clinical value for the patient and that sometimes those values come together. However, attitudes towards genetic testing in children with SNHL have only been investigated regarding testing for *GJB2/GJB6* and demonstrated favorable support for testing. Brunger et al. (2000) [30] found in a questionnaire study where 96/328 responded, that 96% of the parents were positive to genetic testing but that all the respondents had a poor understanding of genetics. This was congruent with the results from Palmer et al. (2009) [31], who showed that parents were positive towards genetic testing, but the ones who received diagnostic test results were more positive than the others. Arnos et al. (2001) [32] emphasized the importance of collaboration between professionals in audiology and genetics.

Here we report a series of eleven patients with profound SNHL who were subjected to cochlear implantation. The purpose of this study is to report the outcome of the genetic testing in the cohort, describe the parental experience and describe our clinical experience of implementing WES and XON array for children with profound SNHL.

2. Materials and methods

2.1Ethics approval

The study was approved by the Swedish Ethical Review Authority (Dnr 2018/282). Both parents of each child included in the study gave informed consent after being provided oral and written information. In cases where one parent did not reside in Sweden and was not a legislated caregiver, verbal consent was accepted through the parent in Sweden. All parents have agreed to publication of the data in this paper.

2.2. Audiological evaluation and inclusion criteria

This is a prospective study of the genetic outcome, as well as the implementation and family experience of WES as a first line of genetic diagnostics in a single tertiary referral center. The study was conducted between December 2018 and June 2020 at the Unit for Audiology at the Department of Otorhinolaryngology at Skåne University Hospital. Children with bilateral SNHL (Pure Tone Average (PTA) > 35 dB hearing level (dB HL) [33] of unknown cause, were offered inclusion in the study provided that their parents were able to understand verbal and written information in Swedish or through an interpreter. Hearing loss was diagnosed according to our standard clinical procedure with OtoAcoustic Emission, Auditory Brainstem Response, Auditory Steady State Responses, electrocochleography, and in one case, pure tone audiometry (Fig. 1) [34].

2.3. Hearing loss panel

The manually curated "HearSeq" list consisting of 179 genes associated with hearing loss is included as Supplementary Table 1 and is developed by members of the research team. The panel consists of nuclear genes related to non-syndromic conditions as well as to syndromes where SNHL can be the presenting symptom. Variants in mtDNA were investigated separately (see below).

2.4. Patient sampling and DNA extraction

Under general anesthesia, in the same session as the hearing assessment or cochlear implantation surgery, venous blood (2–5 ml in EDTA tubes) was collected from each subject. DNA was extracted using standard protocols for the QIAsymphony system (QIAGEN, Germany) and then analyzed in a clinical routine flow as described below.

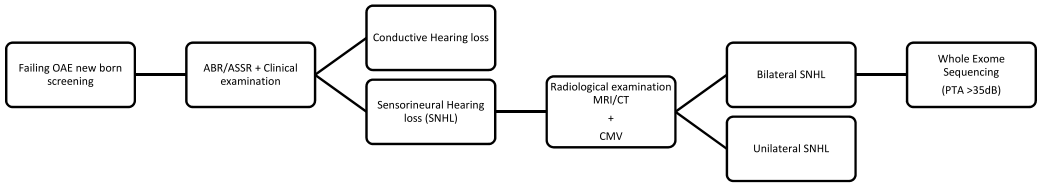


Fig. 1. Flowchart depicting the diagnostic examinations that the children underwent.
OAE, otoacoustic emission; ABR/ASSR, auditory brainstem response/auditory steady-state response; CMV, cytomegalovirus; SNHL, sensorineural hearing loss; PTA, pure tone average.

2.5. XON array

Microarray analysis was completed using the CytoScan XON system (Applied Biosystems, Thermo Fisher, UK) as an exon-level copy number solution. CNVs within the current gene panel were interpreted in the Chromosome Analysis Suite (ChAS) data analysis software, genome version GRCh37.

2.6. Exome sequencing

Exome library preparation with the SureSelect Clinical Research Exome V2 (Agilent Technologies, USA) was completed and the libraries were sequenced on the Illumina NextSeq 500 (Illumina, USA) using 2 × 150 bp pair-end sequencing with an average coverage depth of 80X. Alignment to the GRCh37 reference genome, variant calling, and variant prioritization was completed as in clinical routine using the Burrows-Wheeler Aligner, GATK best practice workflow and an in-house rank model. Technically credible candidate variants within the current gene panel were interpreted and classified according to the ACMG guidelines [26].

2.7. Mitochondrial DNA

Mitochondrial DNA was analyzed for all subjects. Library preparation was completed using Twist Library Preparation EF Kit (product no. 101058, Twist Bioscience, San Francisco, USA) and KAPA HiFi HotStart ReadyMix PCR Kit (product no. 07958927001, Roche, Basel, Switzerland). Sequencing was completed in 2 × 150 cycles on either of the MiniSeq (product no SY-420-1001), NextSeq 550 (product no. SY-415-1002) or NovaSeq 6000 (product no. 20012850) instruments from Illumina (San Diego, USA) after targeted enrichment of mtDNA (Twist Mitochondrial Panel, Twist Bioscience). Coverage of the mitochondrial genome was uneven and did not allow for analysis of CNVs. Variants in mtDNA with a variant allele frequency (heteroplasmy) of >25% in a subject, and a population homoplasmic allele frequency of <0.5% in gnomAD [35] (Hom v.3.1.1) were analyzed for known relationship to hearing loss or primary mitochondrial disease.

2.8. Vestibular examination

The vestibular function of the subjects was tested clinically by an experienced specialized physician at the Unit for Vestibular Disorders at the Department of Otorhinolaryngology, Skåne University Hospital. Clinical examination [36] was combined with video head impulse test (vHIT, Synapsis system, Marseille, France) in nine of the patients, which is an objective and quantitative test of vestibular function. To conduct vHIT in small children parental contribution was required, preferably by both parents. One parent held the child, while the other parent acted as the test object. The examiner stood behind the parent with the child. When the examiner noted that the attention of the child was directed towards the "object parent", the examiner quickly turned the head of the child and the movements of the eyes were recorded. The maneuver was repeated in different directions until enough passed recordings were registered by the system.

2.9. Patient and family experience outcome

A previously reported Patient Reported Experience Measure (PREM) questionnaire on the patient and family experience of genetic investigation was used after modification [37] (original Swedish version, supplement 2; translated English version, supplement 3).

Twelve questions of the original 42 were not relevant to patients with SNHL and 14 questions concerned demographic parameters which, in this study, were extracted from the medical records, and these 26 questions were removed. The 16 remaining questions concerned only the patients with pathogenic, likely pathogenic or VUS findings and were identical to the original version. These questions contained topics about received information and gained knowledge, follow-up, availability and accessibility to the clinic, and care and participation. Five additional questions specifically related to SNHL were presented to the parents of all the subjects, whereas two of the questions "I feel that the genetic testing provided additional value for me and my family" and "I would recommend other families with children with hearing loss undergo this type of testing", concerned the personal additional value of the genetic test. The questions in the questionnaire had four options on an ordinal scale; Disagree, Somewhat disagree, Agree, and Strongly agree. The answers were dichotomized into agreement (answers: Strongly agree or Agree) or disagreement (answers: Somewhat disagree or Disagree). The results are presented as percentage of agreement.

The questionnaire was printed on paper and delivered to patients by mail. The questionnaire was posted in May 2020, two to 14 months after the parents had received the result from the genetic testing. A letter of reminder was posted in cases with absent response. If the questionnaire was still not returned after 1–2 letters of reminder, the parents were asked to fill out the form during a clinical appointment (with access to an interpreter if needed).

To describe the reported patient and family outcomes, we focused on the answers to nine of the most relevant questions in the questionnaire, including four of the original five topics described above: information, follow-up, care and participation, and additional value.

2.10. Creating a network of collaboration, clinical evaluation, and collegial education

Potential multidisciplinary coworkers involved in the diagnostic and habilitation process of children with SNHL were identified. Improving the collaboration within this diverse group of skilled and specialized professionals was part of the implementation process of the new diagnostic work-up. Regular meetings, education in hearing genomics, and continuous discussion between the physicians of the Audiology Department and the physicians of the Clinical Genetics Department were instrumental to the process. Thereafter, members of the research group educated the audiologists of our department about the project and genetics associated to hearing loss.

3. Results

Eleven children aged between five months and eleven years (mean 3.4 years, SD 3.5 years), six females and five males, were included. All had profound SNHL (PTA >70 dB HL) and were in the process of receiving cochlear implants.

3.1. Genetic findings

In total, six of the eleven patients (55%) had variants of interest, and three patients (27%) received a definitive genetic diagnosis. One pathogenic homozygous variant associated with Usher type 1/1B in *MYO7A* was identified, as well as two likely pathogenic homozygous variants in *SLC26A4* associated with Pendred syndrome and *PCDH15* associated with Usher type 1/1F, respectively. Four VUS were found and in three cases there was a potential association with autosomal recessive Usher syndrome (type 1F and 2C). One proband was compound heterozygous for a frameshift variant classified as a VUS on one allele and in *trans* a likely pathogenic deletion on the other allele in the *PCDH15* gene, another proband was homozygous for a VUS in *PCDH15*, and a third child was homozygous for a VUS in *ADGRV1*. The fourth proband had a heterozygous VUS in *TSC* (associated with autosomal dominant hearing loss) that could be disregarded when parental testing demonstrated that the asymptomatic father carried the same variant. The two patients with likely pathogenic variants associated with Usher disease had pathologic findings during electroretinography (ERG) and were thus clinically diagnosed with Usher Disease. The three patients with VUS with a potential association to Usher Syndrome also underwent ERG where no retinal changes were detected. These patients were diagnosed as having isolated non-syndromic SNHL (Table 1). No pathogenic variants in mtDNA were found.

3.2. Vestibular function

Hypofunction of the vestibulo-ocular reflex was found in five of the cases, one unilaterally affected, and implicated loss of vestibular function. Three of these children had variants located in genes associated to Usher Syndrome, one a homozygous pathogenic variant (*MYO7A*, Usher type 1/1B), one a homozygous likely pathogenic variant (*PCDH15*, Usher type 1F) and one a compound heterozygous variant with a frameshift VUS on one allele and a likely pathogenic deletion on the other allele. In the other two subjects with vestibular deficits, there were no pathological genetic findings associated to hearing loss (Table 1).

3.3. Family-reported experience after genetic testing

All eleven participating families answered the questionnaire (5 by mail and 6 during a clinical appointment). The percentage of agreement to the main questions in the questionnaire is presented in Fig. 2. An extended table with results from all topics is presented in Supplementary Table 4.

The parents generally reported that the participation added value to their family (90%) and would recommend that other families participate in this type of genetic testing (100%), including both families with and without a genetic finding related to hearing loss. The participating families who had received information about a genetic finding (n = 5), including two VUS, predominantly answered that their knowledge of the specific hereditary condition had increased (80%), that their own questions about the condition were answered (80%), understood if there was any medical follow-up important for their child (75%), and had confidence in the medical assessment (80%). The verbal information about the genetic condition was found to be clear (80%). However, 60% found it hard to explain the results to other relatives. The parents to the two subjects with VUS had the impression that the genetic finding explained the condition of their child. One of the families with a genetic finding only received written information about the condition. This family was less satisfied with the information, follow-up, and personal care compared to the other parents.

3.4. Multidisciplinary network, professional learning, and impact on the organization

A multidisciplinary approach was critical for successful implementation of genetic diagnostics, primarily between the Department of Audiology and the Department of Clinical Genetics but also including representatives from other departments, depending on the needs of the specific patient (Fig. 3). Educational sessions were organized on a regular basis with knowledge transfer from geneticists to audiology physicians and audiologists regarding general understanding of genomics, and interpretation of specific genetic findings. This resulted in increased knowledge about the genetics of hearing loss amongst all participating professionals.

In cases with pathogenic variants, likely pathogenic variants and VUS with potential involvement of both the auditory and visual sensory systems, the dialogue and collaboration with the Department of Pediatrics and the Department of Ophthalmology became more efficient with earlier and more adequate diagnostic investigations. Five children were referred to the Department of Ophthalmology for ERG due to variants in Usher-related genes. In two cases, pathology related to retinitis pigmentosa were seen and the clinical Usher diagnosis ascertained. In Pendred syndrome (*SLC26A4*), hearing loss can be accompanied by enlargement of the thyroid gland. This child was referred to the pediatric endocrinologist but ultrasound investigation revealed that the thyroid gland was of normal size and a blood test showed normal levels of thyroid hormones. These investigations will be followed up.

During the process of implementing WES in our department, the diagnostic procedure regarding the vestibular function among children with SNHL was optimized. All children with profound SNHL were assessed vestibularly and, in the case of vestibular dysfunction, referred to a physiotherapist. The physiotherapist evaluated the motor development of the child and provided advice to parents regarding practicing the child's mobility as well as discussing expectations. The routines for vestibular training are evaluated in an ongoing study of children with balance disorder.

The families kept close contact with the hearing habilitation team to focus on hearing and speech development. As has been the routine previously, families were offered audio verbal therapy (AVT) as well as education in Swedish sign language. Counselling and parental courses were also offered as part of the hearing habilitation. Children with Usher Syndrome were affiliated with the deaf-blindness team. This provided new challenges for the multi-disciplinary deaf-blindness team with a possibility for earlier habilitation process even prior to the children having developed blindness.

4. Discussion

In this prospective pilot study, we demonstrate the utility of wide genetic testing as the first line diagnostic investigation in pediatric patients with bilateral profound SNHL, and report on the experience of this testing for the families. We show that diagnostic yield is high using a combination of WES and XON-array in these patients, and it is notable that the parents found the testing valuable. We also describe the clinical process of implementation. Increasing the knowledge of genetic diagnostics among audiology physicians and audiologists, collaborating with other clinical departments, and establishing a routine for informing the parents of the results of the genetic analysis in a comprehensive way are central to succeeding with the implementation.

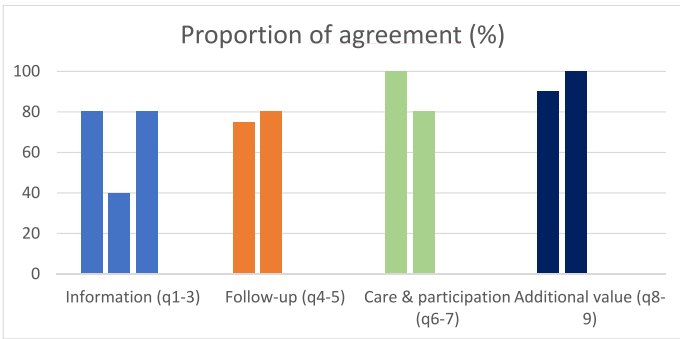
4.1. Genetic findings

This study yielded a relatively high percentage of genetic findings of interest (55%) with a high percentage of variants in Usher related genes

Table 1
Genetic findings grouped according to ACMG criteria and correlated phenotypes.

ACMG class	Gender	Genetic finding	Phenotypic finding	Heredity and genetic family background	Birth country of parents	Hearing function	Time of SNHL diagnose	Hearing rehabilitation	Vestibular function	Other clinical findings
5	female	1. homozygous frameshift-variant in *MYO7A* NM_000260.3(MYO7A):c.6231dup; p.(Lys2078Glufs*50) NM_000260.3(MYO7A):c.6231dup; p.(Lys2078Glufs*50)	AR* Usher syndrome type 1/1B	parents are cousins	Iraq	profound SNHL	1.5 months	bilateral CI**	impaired vestibular response unilateral	impaired vision, retinitis pigmentosa
4	female	2. homozygous frameshift-variant in *PCDH15* NM_033056.3(PCDH15):c.3761dup; p.(Asn1254Lysfs*54) NM_033056.3(PCDH15):c.3761dup; p.(Asn1254Lysfs*54)	Usher type 1F		Sweden	profound SNHL	3 months	bilateral CI	absent vestibular response on V-HIT*** and cVEMP**** bilateral	impaired vision, retinitis pigmentosa
	male	3. homozygous missense-variant in *SLC26A4* NM_000441.1(SLC26A4):c.419C > T; p.(Pro140Leu) NM_000441.1(SLC26A4):c.419C > T; p.(Pro140Leu)	Pendred syndrome		Serbia	progressive, profound SNHL	3 years	bilateral CI	normal vestibular function	LVAS*****
3	female	4. one heterozygous frameshift VUS in *trans* with one pathogenic deletion in *PCDH15* (associated with AR Usher syndrome type 1F) NM_001142769.2(PCDH15):c.4958_4959del; p.(Thr1653Argfs*21) arr[GRCh37] 10q21.1 (55662016_55665482)x1	Non-syndromic SNHL		Sweden	profound SNHL	2 months	bilateral CI	impaired vestibular response bilateral	normal retinal function
	female	5. homozygous missense VUS in *PCDH15* (associated with Usher syndrome type 1F) NM_033056.3(PCDH15):c.2693C > A; p.(Ala898Asp) NM_033056.3(PCDH15):c.2693C > A; p.(Ala898Asp)	Non-syndromic SNHL	1/3 siblings with SNHL	*Middle east*	profound SNHL	5 months	bilateral CI	normal vestibular function	normal retinal function
	female	6. homozygous missense VUS in *ADGRV1* (associated with AR Usher syndrome type 2C) NM_032119.4(ADGRV1); c.13523C > G; p.(Ala4508Gly) NM_032119.4(ADGRV1); c.13523C > G; p.(Ala4508Gly)	Non-syndromic SNHL	parents are cousins	Pakistan	severe SNHL	1 month	hearing aid left ear, CI right ear	normal vestibular function	normal retinal function
	male	7. heterozygous missense VUS in *TNC* (inherited from asymptomatic father) NM_002160.4(TNC):c.3191C > T; p.(Pro1064Leu)			Sweden	progressive, profound SNHL	2.5 years	CI bilateral	normal vestibular function	
Not Applicable	female	8. No finding			Syria	profound SNHL	1.5 months	CI bilateral	absent vestibular response bilateral	delayed motoric development
	male	9. No finding		Twin with nr 10. + another brother with SNHL. Father cousin with the father of the mother.	Pakistan	profound SNHL	3 years	CI right	impaired vestibular response bilateral	
	male	10. No finding		Twin with nr 9. + another brother with SNHL. Father cousin with the father of the mother.	Pakistan	profound SNHL	3 years	CI right	normal vestibular function	
	female	11. No finding			Sweden	progressive and profound SNHL	2.5 years	bilateral CI	normal vestibular function	Atrial septum defect and failure to thrive

*Autosomal Recessive, **Cochlear Implants, ***video Head Impulse Test, ****cervical Vestibular Evoked Myogenic Potential, *****Large Vestibular Aqueduct Syndrome******.



q1: My knowledge of the genetic condition improved.
q2: I understand how to communicate knowledge/information about the genetic condition to my relatives.
q3: The spoken information was clear.
q4*: I was informed about what kind of follow-up is important for my child.
q5: I trust the medical assessment that was made.
q6: I was treated with respect.
q7: My questions were answered.
q8: I feel that the genetic testing provided additional value for me and my family.
q9: I would recommend other families with children with hearing loss to go through this type of testing.
*Answers only from 4/5 families.

Fig. 2. Family reported outcome from the follow-up questionnaire after the study of genetic testing of patients with SNHL. Results in proportion of agreement in % (answer options: "Strongly agree" or "Agree" compared to options "Somewhat disagree" or "Disagree") to nine of the questions included in the questionnaire. The first seven of these questions addressed only those families with a child with a genetic finding, whereas two of the families with VUS responded and one did not (n = 5). The last two addressed all participating families (n = 11). The attitude was predominantly positive towards the included question topics (Information, Follow-up, Care and participation, and Additional value). Some of the families struggled to explain the genetic condition to other family members. The families generally reported that the participation added value, regardless of benign or pathogenic outcome, and would recommend this type of genetic testing to others with SNHL.

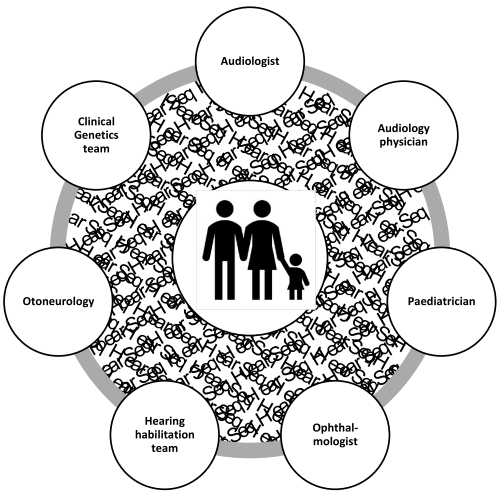


Fig. 3. A multidisciplinary approach and a close relationship between colleagues in a variety of medical fields is a key factor for success with implementation of massive parallel sequencing technologies.

(45%), and three patients (27%) had pathogenic or likely pathogenic variants consistent with a clear genetic diagnosis. Based on the literature, around 20% of individuals with congenital SNHL are expected to have variants on *GJB2/GJB6* gene [9,38], but we found no *GJB2* or *GJB6* variants in our analyses, probably a coincidental effect due to the relatively small number of patients.

VUS accounted for more than half of the genetic findings in our study and are not considered clinically relevant until more information is gathered. However, we argue that if there is a VUS in a gene related to a syndrome where other symptoms may co-occur, referrals should be offered for further investigation. The pathogenic deletion in *PCDH15* in patient 4 (Table 1) was detected with XON-array, whereas all other variants were detected with WES.

The proportion of genetic findings in our study is in line with a recent genetic study with massive parallel sequencing (MPS) on patients with SNHL in Spain, Cabanilla et al. (2018) [39], which showed a diagnostic ratio of 42% (n = 21/50) excluding VUS when using a gene panel of 199 genes. Prior to the study, patients with known causes of SNHL including variants in the *GJB2/GJB6* genes were excluded and they reported a diagnostic genetic yield of between 50 and 60% for the study subjects. The same applies to another study from Australia, Drownie et al. (2019) [13], which had a diagnostic yield of 56% (n = 59/106) when examined in children with moderate to severe SNHL with WES and microarray. Similar to this study, they accounted for VUS with favorable pathogenicity, namely when the variants were in trans and had no conflicting benign evidence.

Finding variants associated with Usher and Pendred syndromes is expected since these mutations are considered relatively common. Usher Syndrome (type 1–3) is expected in around 1 of 20 cases [40] of children with SNHL compared to the present cohort where variants associated to Ushers Syndrome were found in two of eleven cases. Pendred Syndrome on the other hand has been estimated to account for around 10% of the cases with SNHL [41], thus one in eleven as shown here was expected.

As shown above, the ratio of genetic findings in the current study is in line with what the literature has shown so far, but in our cohort surprisingly many variants commonly associated with Usher were found and variants in *GJB2/GJB6* were missing. The patients in our cohort have a diverse ethnicity, with only four patients with Swedish origin. This can affect the expected background variant frequency.

Pathogenic variants in mtDNA may cause SNHL, either syndromic or non-syndromic, thus we propose that investigation of mtDNA in addition to nuclear DNA is an important part of the genetic analysis of hearing loss. Among these eleven subjects, no mtDNA variants linked to hearing loss were found. Copy number variation in mtDNA could not be assessed due to uneven coverage of the mitochondrial genome, hence we were not able to exclude mtDNA deletions, a well-described mtDNA pathology associated with hearing loss [42]. Our research team intends to further investigate the use of genetic testing of mtDNA in children

with hearing loss.

4.2. Vestibular function

In line with previous studies [43–45] almost half of the patients (45%) in the present study were diagnosed with vestibular dysfunction including two patients with Usher syndrome. The high prevalence for vestibular dysfunction in children with hearing loss is well-known and related to the anatomical proximity and the phycological similarities between the cochlea and the vestibular end-organ [45–47]. Melo et al. [48] showed that children with SNHL caused by post-natal meningitis or prematurity were more likely to suffer from vestibular dysfunction, whereas the prevalence of vestibular dysfunction in children with genetic caused non syndromic SNHL needs further investigation. Studies have shown that children with SNHL and vestibular dysfunction have an impaired balance [46,49], which affect their motor skills [50] and also, their ability to attend social and sport activities and thus, all children with SNHL, irrespective of genetic finding, should undertake a vestibular assessment. Our patients were examined thoroughly and their vestibular function assessed, but the important part; to engage physiotherapists and offer vestibular training programs [48] to children with impaired balance has been challenging to accomplish and habilitation of this deficit still needs more attention in our clinic.

4.3. Family experience

The parents in this study were generally positive to genetic testing and would recommend the test regime to other families with a child with SNHL. In cases where the genetic finding led to referrals and examination in other departments (ophthalmology and pediatrics) with a directed question instead of a standardized referral, the evaluation of the child and the following habilitation were more precise. Even in cases where there were no pathologic genetic findings, the parents experienced a personal gain of the test, consistent with the literature concerning genetic diagnostics in rare diseases [29] and *GJB2/GJB6* [30, 31]. The questionnaire used in this study enabled nuanced and trustworthy answers regarding patient satisfaction [37], but the difficulty is to determine if diagnosing SNHL on a molecular level adds personal value to the families or if it is something else in the process that provides value. The personal benefit from sequencing technologies for families with children with SNHL needs further, quantitative as well as qualitative, evaluation.

One family who received only written information reported less satisfaction regarding information and knowledge, follow-up, and personal care. The study was not designed to compare modes of providing information, but the views expressed by this family were in line with our experience that it is important to inform patients and families about test results during a clinical visit. The fact that the majority of the parents struggled to convey their newly gained knowledge about the genetic finding to other relatives further indicates that the way in which information is provided needs to be considered as an important part of implementing extended genetic testing in the clinic. There may be a need for individual adjustments in the communication strategies that are used, a follow-up appointment or extended genetic counselling to address this.

Two of the three families of children with VUS had interpreted their result as a confirmed genetic diagnosis, clearly illustrating the difficulties of informing families about genetic testing results. The reason for these misunderstandings were not investigated, but this further emphasizes the importance of considering how results are interpreted and communicated to patients and families, especially when there is ambiguity about the clinical relevance of the results.

4.4. Implementing new technology and the impact on multidisciplinary networks, professional learning, and the organization

A major challenge with the implementation was dissemination of knowledge throughout the multidisciplinary professional team. Groups of professionals within the same organizational unit, such as audiologists, were easier to inform compared to teachers of special needs, psychologists, social workers, and speech and language therapists, who were all situated at other locations. Most health professionals have limited previous knowledge about genetics but still need to respond to questions from the parents.

The present report focuses on variants associated with SNHL, but the actual genetic sequencing was not selective for the genes on the gene panel. The investigation may therefore give rise to secondary (incidental) findings not related to SNHL. Some laboratories report on secondary findings while others do not [51], and it is advisable to ensure that there are routines for handling this as well as to prepare parents before their consent that genetic evaluation of the child might reveal incidental findings.

4.5. A genetic analysis is easy to conduct, but not always obvious to interpret

When doing genetic testing, variants of unknown significance, VUS, will be found, as was the case in three patients in this study. Sometimes further testing of relatives to see if the variants segregate with pathology, or additional clinical examination, for example ERG, can provide further guidance. Again, a multidisciplinary approach including not only geneticists and otorhinolaryngologists but also ophthalmologists and pediatricians is often required to appropriately handle all aspects of a finding where there is uncertainty of its clinical relevance.

When variants are found in genes correlated to a syndrome, the information process can be sensitive. In variants associated with Usher Syndrome, further examination with ERG were completed prior to a conclusion of Usher diagnosis. Our patients did not receive the Usher diagnosis unless there were signs of retinitis pigmentosa. This does not mean that the found variants are obligate irrelevant, since many variants in genes associated to Usher syndrome is also associated with isolated hearing loss. Thus, the diagnosis was based on phenotype rather than genotype and in some cases the genetic finding created unnecessary anxiety. It needs to be emphasized that patients and the parents of the present study were referred to a clinical geneticist if genetic counselling was requested. According to Swedish law, prenatal diagnostics is only available when a known inherited pathogenic or likely pathogenic variant is present. Arguably, prenatal diagnostics is ethically questionable in individuals with isolated SNHL [32,36], but it is important to be humble for the reasoning of others.

4.6. Clinical experience from the view of the physician of audiology

We recognized that adding extra time to clinical appointments for verbal information was valuable, as the abilities of parents to receive and process information varied. Efforts were made to provide information to both parents, but this was difficult when only one parent accompanied the child to the appointment. Another challenge was to ensure that parents had properly understood the information given through an interpreter. If genetic testing did not reveal any mutations, a telephone call could replace the appointment in cases of easy communication, but not in cases with a pathological finding.

4.7. Strengths and limitations

Family experience from whole exome sequencing in children with SNHL has not been reported before, although previous studies have shown positive attitudes to single gene tests. We have a response rate of 100% and the data, although from a limited sample size, may provide

important information for professionals in audiology as this kind of testing becomes more widespread.

This study can act as a guide to implementation of genetic testing at a tertiary referral center for audiology, demonstrating the necessity for a multidisciplinary approach and efficient information and knowledge dissemination in the organization.

The small number of patients is a limitation of this study but does not obscure its prime purpose, implementation of genetic investigation of SNHL. As a consequence of the rapid development of genetic technologies, the methodologies used in this study had, by the time of submission of this article, already been replaced by WGS, a technique that at once replaces all three methods used in this study (WES, XON-array and targeted mtDNA sequencing). From the perspective of the audiology professional, the distinction between these methods is not critical for the interpretations of results and the care of the patient.

4.8. Clinical implications

Implementing MPS as first line of molecular diagnostics for children with SNHL will not only give a higher diagnostic yield but also identify syndromes associated to SNHL at an early stage. If associated symptoms can be detected earlier and/or prevented, earlier habilitation efforts can be initiated.

5. Conclusion

The new genetic test regime added diagnostic value for children with SNHL. The parents found the genetic testing valuable. Children with an early genetically verified diagnosis could be provided with more accurate evaluation and support. Great care should be taken to thoroughly inform parents about the genetic test result. Collaborations between departments were intensified and knowledge of genetic causes of sensorineural hearing loss were improved among the staff.

Usher syndrome

SNHL and vision loss cause by retinitis pigmentosa associated with variants in a variety of genes, including *MYO7A*, *ADGRV1* and *PCDH15* genes.

Type 1: Severe to profound SNHL from birth, progressive vision loss in childhood and vestibular abnormalities.

Type 2: Mild to severe SNHL mainly in high frequencies from birth and progressive vision loss in adolescence or adulthood.

Type 3: SNHL and vision loss in late childhood or adolescence and sometimes vestibular abnormalities.

Pendred syndrome

Progressive SNHL in early childhood with Enlarged Vestibular Aqueduct (EVA) and abnormal cochlea accompanied by enlarged thyroid gland and vestibular abnormalities caused by variants in *SLC26A4* gene.

Declaration of competing interest

None of the authors declare any conflicts of interest, financial or otherwise. This study was funded by ENT-department, Skåne University Hospital, Lund;Acta Oto-Laryngologica Foundation; Hörselforskningsfonden; Södra Sjukvårdsregionen Regionmedel, the Swedish Royal Physiographic Society; The Magnus Bergvall Foundation; Fredrik and Ingrid Thuring's Foundation; The Lars Hierta Memorial Foundation and The Swedish Society of Medicine.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijporl.2022.111218.

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Paper III

Original Research

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Diagnostic Yield and Genetic Variation in 85 Swedish Patients with Mild to Profound Hearing Loss Analyzed by Whole Genome Sequencing

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Abstract

Importance. The genetic variation in patients with sensorineural hearing loss (SNHL) in the Nordic countries has not been previously reported.

Objectives. The aim was to describe the genetic variation in a Swedish population and identify factors in favor of a high diagnostic yield.

Design. This was a prospective cohort study. Children with bilateral SNHL and adults with bilateral SNHL and clinically suspected genetic SNHL underwent genetic testing. A gene panel with ~200 genes was applied on whole genome sequencing (WGS) data. Variants were classified according to American College of Medical Genetics and Genomics criteria. Personal health data were extracted from medical records.

Setting and Participants. Eighty-five patients (aged 0-73 years) from Lund and Örebro University Hospitals, 2 tertiary referral centers for audiology in Sweden, with mild to profound SNHL.

Results. In almost half (45%, n = 38) of the cases, a genetic cause was identified across 24 different genes. Eleven cases had syndromic hearing loss. A majority (n = 57) had prelingual onset (<2 years) of SNHL and most of them had moderate-to-profound hearing loss (n = 52). Prelingual onset was associated with higher yield than postlingual onset (OR 6.3, 95% CI 2.1-19.0). In patients with moderate—profound prelingual SNHL, the diagnostic yield was 60% (n = 31/52).

Conclusion. This is the first reported cohort of hearing loss patients undergoing genetic testing with WGS from a Nordic country. Early onset of hearing loss favored a higher diagnostic yield than postlingual, and a genetic cause was found in a majority of cases in patients with prelingual, moderate-to-profound SNHL.

Keywords

genetic hearing loss, whole genome sequencing, pathological variants, prelingual SNHL, diagnostic yield

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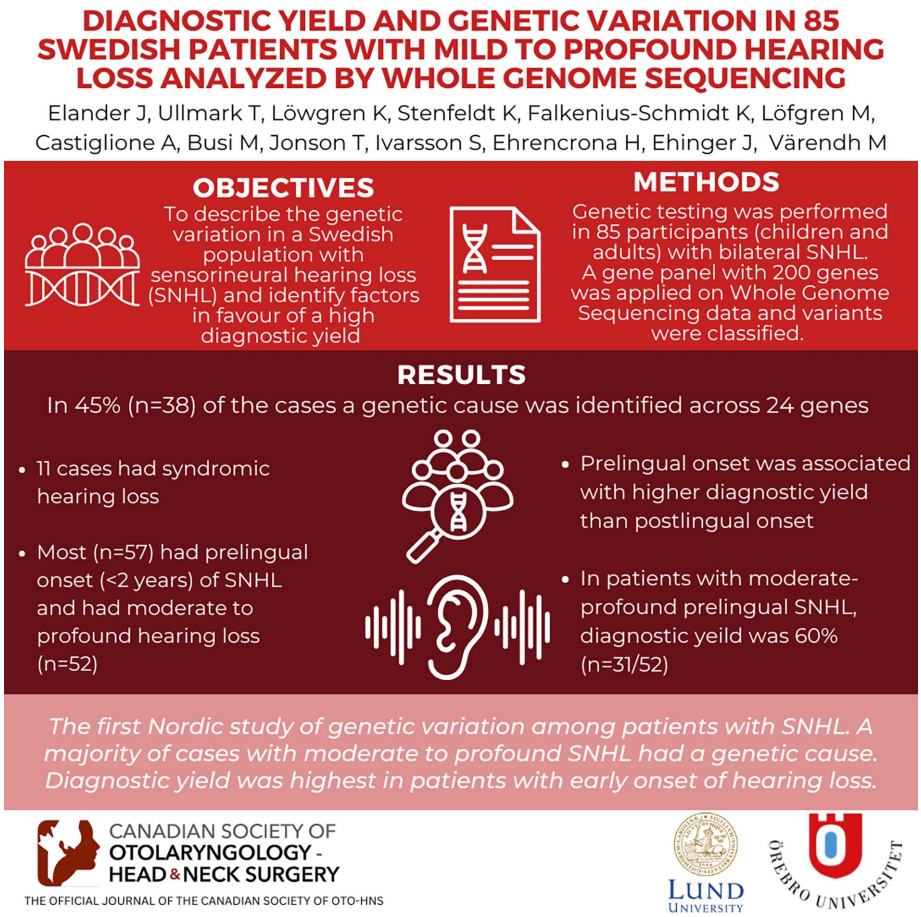
Graphical Abstract



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Key Message

- The first Nordic study of genetic variation among patients with sensorineural hearing loss (SNHL).
- A majority of cases with moderate-to-profound SNHL had a genetic cause.
- Diagnostic yield was highest in patients with early onset of hearing loss.

Introduction

Genetic Investigation of Hearing Loss

Sensorineural hearing loss (SNHL) is the most common sensory deficit in newborns and the prevalence increases with age.[1] Genetic variation is the most prevalent cause of SNHL in children, both in isolated and syndromic cases.[2-10] The most frequent syndromes, with SNHL as part of the symptom combination, are Pendred syndrome, with inner ear malformations and goiter[11] and Usher syndrome, with visual loss due to retinitis pigmentosa (RP) and vestibular impairment.[12] Usher syndrome is related to variants in 9 confirmed causative genes, and in several genes (such as *MYO7A*, *USH1C*, *CDH23*, and *PCDH15*), either related to isolated SNHL or concomitant progressive visual loss with RP. RP in young children is investigated with electroretinography (ERG) under general anesthesia.[13-16]

In recent years, massive parallel sequencing is increasingly used for investigating SNHL with unknown cause[17] (exome or genome sequencing). A gene panel including genes relevant for hearing is then applied, to filter the vast amount of data and facilitate the analysis.

Hearing loss is defined by WHO, based on hearing threshold on a pure-tone audiogram, as >20 dB hearing loss (HL) based on four-frequency (0.5, 1, 2, and 4 kHz) pure-tone average (4fPTA) and varies from mild to profound.[18] Hearing loss can be conductive or sensorineural. Age of onset of hearing loss is often defined in relation to normal age for development of spoken language and can broadly be classified as pre- or postlingual.

Consanguinity within the family is uncommon in contemporary Swedish society, but in some immigrant communities, partnership with a cousin or other relative is within the cultural norm. This is relevant for SNHL as the risk of autosomal recessive traits being biallelic is increased in families with a common genetic background. According to the official statistic governmental agency, Statistics Sweden,[19] 30% of the inhabitants in the regions of Sweden from which the current cohort was recruited (Skåne and Örebro) are either born, or have both parents born in a foreign country. Country of origin is not defined in this register.

In a study from Belgium in 2023, the diagnostic yield was 39% in 238 probands with congenital or late onset bilateral SNHL.[20] Similar findings were reported from the Netherlands in 2017 with 33.5% diagnostic yield in 200 probands with hearing impairment.[7] In a study from Germany in 2022, the diagnostic yield was 25%, but in this study, a large proportion of adults with hearing loss was included.[9] To our knowledge, no similar studies have been presented from Sweden or the Nordic countries.

The aims of this study were to describe (i) the genetic variation related to SNHL in a Swedish population and (ii) to identify the patient groups who would most benefit from genetic testing, in terms of diagnostic yield, depending on SNHL severity and time of onset.

Materials and Methods

As part of a clinical visit, probands with bilateral mild-to-profound SNHL (hearing threshold >25 dB HL) with unknown cause, were enrolled between July 2020 to December 2022 at 2 tertiary audiological referral centers in Sweden (Örebro University Hospital and Skåne University Hospital in Lund). Of 111 consented subjects, 85 provided venous blood from which DNA was extracted and whole genome sequencing (WGS) performed. Only data from the participants who underwent WGS were analyzed. Fifty-one patients were recruited from Skåne University Hospital and 34 patients from Örebro University Hospital. The patients from Lund had prelingual SNHL in 73% (n = 37/51) and from Örebro 62% (n = 21/34) of the cases. Children with an obvious clinical appearance of a syndrome were referred to a pediatrician for assessment and investigation and were not included in the study.

The study was approved by the Swedish Ethical Review Authority (Dnr 2018/282). After verbal and written information, the legal caregivers for children, or the proband, if they were adults, provided written consent.

Audiological Testing

The audiological examination was performed according to age-appropriate clinical procedures. Otoacoustic emission, auditory brainstem response, auditory steady-state response, and electrocochleography as well as subjective methods, visual reinforcement audiometry, conditioned play audiometry, or conventional pure-tone audiometry, when possible (in older children and adults), were used. SNHL was graded as mild (21-40 dB HL), moderate (41-60 dB HL), severe (61-80 dB HL), or profound (>80 dB HL) based on 4fPTA according to the WHO definition from 1991.[21] The updated definition recommended by the Global Burden of Disease Expert Group on Hearing Loss[18] is not validated for children.[22] SNHL diagnosed before 2 years of age was defined as prelingual hearing loss.

Demographic and Clinical Data

A clinical research form including sex, age at SNHL diagnosis, degree and type of hearing loss, number of siblings with and without SNHL, and parental hearing loss status was completed when referral was made for genetic testing. Complementary personal data included parents' self-reported country or region of birth, consanguinity, comorbidity, and

vestibular findings; clinical examination and video head impulse test as previously described in Elander et al[23] were extracted from the medical records.

WGS and Hearing Loss Panel

DNA was sequenced (NovaSeq 6000, Illumina, USA) with an average read depth of 30×. The resulting files were run using an in-house bioinformatic pipeline (https://github.com/Clinical-Genomics-Lund/nextflow_wgs). Analysis of the mitochondrial genome (mtDNA) was added to the pipeline in May 2021. Variants [single-nucleotide variants, indels, copy number variants (CNVs)] were scored and ranked, based on the attributed information and uploaded to the main interpretation tool Scout (https://clinical-genomics.github.io/scout). Variants within genes in the current gene panel (HearSeq) as well as in mtDNA were interpreted in Scout, with support from Alamut (https://www.sophiagenetics.com/platform/alamut-visual-plus), Integrative Genomics Viewer (https://software.broadinstitute.org/software/igv) and locally developed visualization tools (https://github.com/Clinical-Genomics-Lund/gens). A genomewide CNV-analysis was performed to detect any larger CNVs. Furthermore, pathogenic variants in the ClinVar database outside the gene panel were assessed and reported if relevant for the clinical indication. For protein prediction for missense variants Align GVGD, MutationTaster, Polyphen-2 and SIFT were used. The ranking model included scores from CADD (for missense variants and indels), Polyphen and SIFT (for missense variants), and MaxEntScan (for splicing variants). All variants were classified according to the American College of Medical Genetics and Genomics standards and guidelines for interpretation of sequence variants.[24-26] In this report, if not explicitly specified, likely pathogenic (class 4) and pathogenic (class 5) variants are collectively described as pathogenic variants (PVs). Variants of uncertain significance (VUS) were not regarded as sufficient for diagnosis, even in autosomal recessive compound heterozygous cases with 1 PV in trans. The classification of inheritance pattern, autosomal recessive or autosomal dominant, was based on genetic diagnosis.

The gene panel HearSeq, developed at Skåne University Hospital in Sweden, was used and updated twice during the study. The initial gene panel (version 4.0) included 179 genes, whereas the next version 6.0 (updated 05-07-2021), included 196 genes and version 7.0 (updated 20-09-2022) included 201 genes.[27] The HearSeq panel includes genes related to isolated hearing loss and genes related to syndromic SNHL, where SNHL can be the first presenting symptom (Supplemental Table 1).

Statistical Analyses

Descriptive analyses of the data were performed for sex, degree of SNHL, age of onset of SNHL, origin, and heredity. Chi-square test was used to identify associations between genetic diagnostic yield and subgroups, both regarding degree of hearing loss and time of onset. The analysis was complemented with multinominal logistic regression analysis with profound SNHL as reference, to create a model of relationship between the predictor variable and the subgroups, and to analyze if time of onset was a confounder. The SNHL subgroups were compared separately with Fisher's exact test. All analyses and calculations were executed in IBM SPSS Statistics, USA (version 29.0.0.0).

Results

Demographic Data

Fifty-seven probands (67%) had a prelingual SNHL. Age of onset of SNHL diagnosis varied from neonatal to 30 years of age. There was a preponderance of females (n = 51) versus males (n = 34). Degree of SNHL varied from mild (14%) to moderate (28%), severe (11%), and profound (47%) among the patients who underwent genetic testing (Figure 1).

Most of the patients were otherwise healthy (68%). The dominating comorbidity was vestibular dysfunction (11%). Intellectual disability was found in 6% of the cases (Table 1).

The majority had parents born in Sweden (58%, n = 49), while 27% (n = 23) had parents born in the Middle East (Syria = 6, Iraq = 6, Lebanon = 2, Turkey = 2, Afghanistan = 1, Palestine = 1, Kurd = 1, Arabic spoken, but country not specified = 3). In 1 family, 1 parent originated from Jordan and the other parent from Lebanon. Twelve percent of probands (n = 10) had parents from Europe outside of Sweden, and in 3 cases, parents originated from elsewhere in the world (India, China, and Eritrea). One child had 1 parent originating in Scandinavia and the other parent from North Africa. Consanguinity was not systematically documented but reported when documented in the medical records. More than half of the parents from the Middle East (n = 12) were documented relatives and in 10 cases, cousins. Parents of 1 child from East Africa were cousins.

Genetic Diagnostic Yield in Relation to Degree and Time of Onset of Hearing Loss

The overall genetic diagnostic yield was 45% with PVs reported in 38 probands and found in 24 different genes (Figure 2).

Twenty-seven patients (32%) with identified PVs had isolated SNHL. Eleven (13%) had syndromic SNHL. A vast majority of the patients with PVs (n = 34) had an autosomal recessive inheritance pattern and 21 were homozygous. The diagnostic yield in mild, moderate, severe, and profound SNHL groups was 8%, 42%, 67%, and 53%, respectively (Figure 3A).

Chi-square test (linear-by-linear association) showed a significant difference in genetic diagnostic yield between SNHL subgroups ($P < .01$) and time of onset ($P < .001$). Logistic regression analysis, with verified genetic diagnosis as a

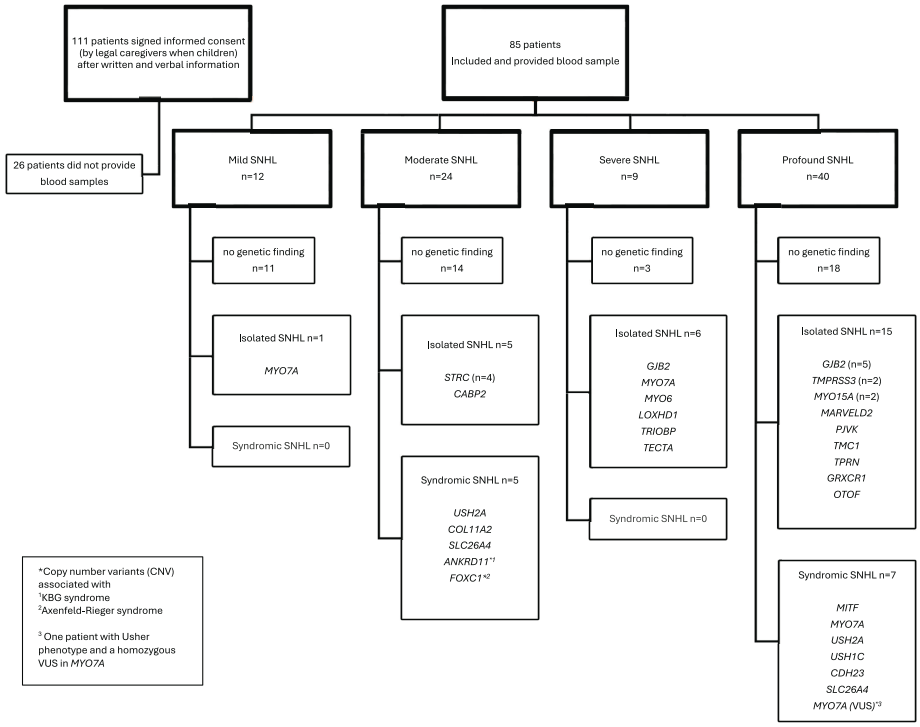

Figure 1. Flowchart showing all patients in the study and the genetic result at the gene level grouped by severity of sensorineural hearing loss (SNHL).

dependent variable and SNHL subgroups as an independent variable with profound SNHL as reference, revealed a significant low odds ratio, OR = 0.08 (95% CI 0.01-0.7) (Figure 3A) in the mild SNHL group compared to the profound SNHL group. For moderate (OR = 0.6, 95% CI 0.2-1.8) and severe (OR = 1.8, 95% CI 0.4-8.3) SNHL, the odds were not significantly different from the profound hearing loss group. The odds ratio for a prelingual onset were high (OR = 6.3, 95% CI 2.1-19.0). When SNHL subgroups were adjusted in the regression model for time of onset, the difference in genetic findings between mild SNHL and the reference group was no longer statistically significant (OR = 0.1, 95% CI 0.01-1.1). However, regarding prelingual onset, a significantly higher diagnostic yield (OR = 6.6, 95% CI 1.9-22.6) remained after adjustment (Table 2B). In addition, the different subgroups were compared separately using the Fisher's exact test, revealing a significant difference between mild and severe SNHL ($P = .02$) and between mild and profound SNHL ($P = .01$), respectively (Table 2A).

Prelingual SNHL dominated with 67% versus 33% with a postlingual onset. A verified genetic diagnosis was found in 54% (n = 31/57) of the probands with prelingual, and in 25% (n = 7/28) of the probands with postlingual SNHL (Figure 3B). In patients with moderate-to-profound prelingual hearing loss the diagnostic yield was 60% (n = 31/52).

Mild-to-Profound SNHL

One child (nr 1) (8%) out of 12 patients with mild SNHL had compound heterozygous PVs in *MYO7A*, but no retinal changes could be identified at 7 years of age (Table 3A). This child had a younger sibling (not regarded as proband) with similar hearing loss, who had the same, previously described,[28,29] compound heterozygous variants in trans. The child is not yet tested with ERG.

In patients with moderate SNHL a genetic diagnosis was identified in 42% (n = 10/24), whereas 5 had isolated SNHL with PVs in *CABP2* (n = 1) and in *STRC* (n = 4), and 5 were syndromic-associated variants. One of the variants affecting *STRC* (nr 13) was a homozygous deletion covering both *STRC* and *CATSPER2*; in males, this would have caused a combination of deafness and infertility,[30] but this was a female patient, and the hearing loss was thus not syndromic. Syndromic-associated variants resulted in Usher syndrome type 2A, Stickler syndrome type 3, and Pendred syndrome (*USH2A*, *COL11A2*, and *SLC26A*4), a frameshift variant in

Table 1. Demographic Variables.

Degree of hearing loss	Mild (n = 12)	Moderate (n = 24)	Severe (n = 9)	Profound (n = 40)	Total (n = 85)
Sex (female:male), n	8:4	19:5	5:4	19:21	51:34
Genetic diagnosis, total, n (%)	1 (8%)	10 (42%)	6 (67%)	21 (53%)	38 (45%)
Genetic diagnosis, prelingual, n (%)	0	8 (33%)	5 (55.5%)	18 (45%)	31 (36%)
Genetic diagnosis, postlingual, n (%)	1 (8%)	2 (8%)	1 (11%)	3 (7.5%)	7 (8%)
Age at onset of SNHL					
Median years (min.-max.)	1.5 (0-16)	0 (0-15)	2.25 (0-5)	0 (0-30)	0 (0-30)
Prelingual, <2 years, n (%)	5 (42%)	15 (63%)	5 (56%)	32 (80%)	57 (67%)
Postlingual					
Preschool age, 2-5 years, n (%)	2 (17%)	3 (13%)	4 (44%)	4 (10%)	13 (15%)
School age, 6-12 years, n (%)	0	2 (8%)	0	3 (7.5%)	5 (6%)
Teenager, 13-19 years, n (%)	2 (17%)	2 (8%)	0	0	4 (5%)
Young adult, 20-29 years n (%)	1 (8%)	0	0	0	1 (1%)
Adult, 30 years, n (%)	2 (17%)	2 (8%)	0	1 (2.5%)	5(6%)
Age when genetic test					
Median years (min.-max.)	11 (1-44)	10.5 (0.6-73)	4.5 (0.5-44)	3 (0.2-47)	6.75 (0.2-73)
<2 years when tested, n (%)	5 (42%)	5 (21%)	3 (33%)	17 (43%)	30 (35%)
≥2 years when tested, n (%)	7 (58%)	19 (79%)	6 (67 %)	23 (57%)	55 (65%)
Origin, n (%)					
Sweden	8 (67%)	18 (75%)	3 (33%)	20 (50%)	49 (58%)
Rest of Europe	1 (8%)	0	3[1] (33%)	6 (15%)	10[1] (12%)
Middle East	2 (17%)	5 (21%)	2 (22%)	14 (35%)	23 (27%)
Other parts of the world	1 (8%)	1 (4%)	1 (11%)	0	3 (3%)
Comorbidity, n (%)					
Healthy	9 (75%)	13 (54%)	9 (100%)	26 (65%)	58 (68%)
Vestibular symptoms or findings	1 (8%)	3 (13%)	0	5 (12.5%)	9 (11%)
Intellectual disability	0	3 (13%)	0	2 (5%)	5 (6%)
Heart disease	0	1 (4%)	0	1 (2.5%)	2 (2%)
Visual impairment	0	0	0	2 (5%)	2 (2%)
Other symptoms and anomalies[2]	2 (17%)	4 (17%)	0	4 (10%)	10 (12%)

Abbreviation: SNHL, sensorineural hearing loss.
[1]Including a child with one parent from Norway and one from Tunisia.
[2]Renal dysfunction, asthma, migraine, growth hormone therapy, facial anomalies, Mondini malformation and large vestibular aqueduct syndrome (LVAS), Down syndrome, suspected Cogan´s syndrome.

ANKRD11 resulted in KBG syndrome (short stature, facial and skeletal anomalies, intellectual disability, and macrodontia syndrome) and a large deletion including *FOXC1* resulted in Axenfeld–Rieger syndrome (Table 3B). In the 2 cases with PVs in *CABP2* and *COL11A2*, the parents were relatives.

In patients with severe SNHL, PVs were identified in 6 out of 9 cases (67%) and were related to isolated SNHL (*GJB2*, *TECTA*, *MYO7A*, *MYO6*, *LOXHD1*, and *TRIOBP*). The child with a homozygous variant in *MYO7A* (nr 38) was examined with ERG, without retinal changes, before 2 years of age. Three of the probands (*TECTA*, *LOXHD1*, and *TRIOBP*) (nr 37, 40, 41) had parents who were cousins (Table 3C).

Among patients with profound SNHL, a genetic cause was identified in 21/40 cases (53%). Of the 32 probands with prelingual onset of hearing loss, 59% (n = 19) received a genetic diagnosis. Isolated SNHL was identified in 15 cases [*GJB2* (n = 5), *TMPRSS3* (n = 2), *MYO15A* (n = 2), *TMC1*, *TPRN*, *OTOF*, *MARVELD2*, *PJVK*, and *GRXCR1*] and a syndromic SNHL genetically detected in 6 cases. The dominating syndrome was Usher, found in 4 cases (*MYO7A*, *USH1C*, *USH2A*, and *CDH23*). The remaining patients with syndromic SNHL had Pendred (*SLC26A4*) and Waardenburg syndrome type 2A (*MITF*). Parents were documented to be relatives in 3 cases (Table 3D).

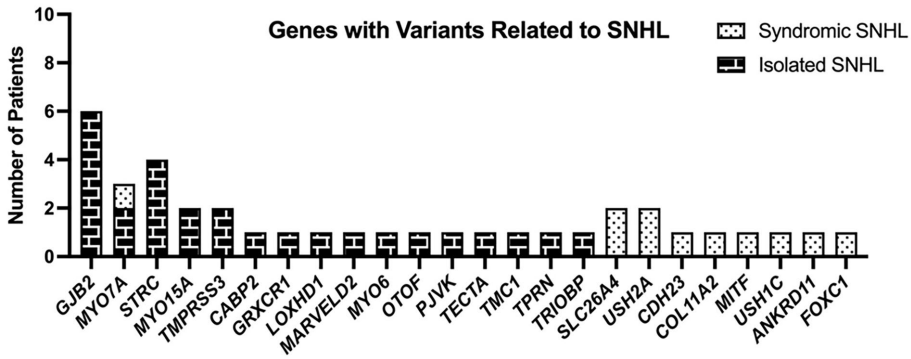

Figure 2. Genes with variants related to sensorineural hearing loss (SNHL).

Usher Syndrome and Related Variants

The most common syndrome was Usher syndrome (n = 6). Genetic variants were found in *USH1C*, *USH2A* (n = 2), *MYO7A*, *CDH23*, and a VUS in *MYO7A*. The patient (nr 64) with the VUS was clinically diagnosed with Usher syndrome and despite the absence of a definite genetic diagnosis regarded as having Usher syndrome and reported here. In addition, 2 cases with variants in *MYO7A* (nr 1 and nr 38), and 1 case with VUS in an Usher-related gene (*PCDH15*) (nr 79, Supplemental Table 2) underwent ERG, and no retinal changes were detected. Nevertheless, ophthalmological re-examinations were planned as the children grow older to monitor whether RP develops over time. The 2 cases with variants in *MYO7A* were regarded as having mild and severe isolated hearing loss, respectively, reported in Table 3, and the VUS in the last case, with no additional clinical symptom, was regarded as not being clinically relevant and thus, not reported in Table 3.

Inheritance Pattern and Consanguinity

Autosomal recessive (AR) inheritance pattern was seen in 90% (n = 34/38) of the cases with PVs, and in all but 1 case (95%) with isolated SNHL. The AR PVs were homozygous in 20 cases. In the group with self-reported consanguinity (n = 13), homozygous variants were seen in 10 cases. Among the other 10 with homozygous variants, 4 had parents originating from Sweden, 1 from the Middle East and 1 each from Turkey, Macedonia, Serbia, Albania, and Poland. In the group with compound heterozygous variants, 11 had parents originating from Sweden, 2 from the Middle East (Syria, Lebanon), and 1 from Kosovo. Of the patients with a verified genetic diagnosis, 39% (n = 15/38) were multiplex families, with one (n = 9) or more (n = 6) first-degree relatives, siblings or parents, with hearing loss. Among patients where we did not find a genetic explanation, 21% (n = 10/47) had one (n = 5) or more (n = 5) first-degree relatives with hearing loss. Of the 4 with autosomal-dominant inheritance patterns, 1 had a parent and a sibling with hearing loss.

Discussion

Main Results

In our prospective cohort study, PVs were found in 24 genes, and the diagnostic yield in the entire cohort was 45%. Probands with prelingual moderate-to-profound SNHL were likely to receive a genetic diagnosis, with a diagnostic yield of 60% (n = 31/52).

In total, 8 PVs in this cohort were CNVs, showing the importance of including a copy number analysis in genetic diagnostics.

The Value of Genetic Testing, in Relation to Diagnostic Yield and Onset

In this cohort, patients with a prelingual SNHL were more likely to have an identifiable genetic cause than individuals with postlingual SNHL. The association between prelingual SNHL and a genetic diagnosis was significant and also an important confounder when comparing subgroups of children with SNHL of various degree. The preponderance of genetic findings among patients with prelingual hearing loss has been described previously, for example, in a Dutch population[7] and in a recent German publication as previously discussed.[9]

The classification of pre- and postlingual hearing loss involves some uncertainty as neonatal screening with Transient Evoked Otoacustic Emissions does not detect mild SNHL, SNHL isolated to high or low frequencies and auditory neuropathies. Nevertheless, the main part of the probands in our study had prelingual hearing loss. There is a risk of ascertainment bias, where the genetic background of prelingual moderate-to-severe SNHL is more thoroughly investigated. In the postlingual population, there might be other genetic/polygenic or covariable factors involved.

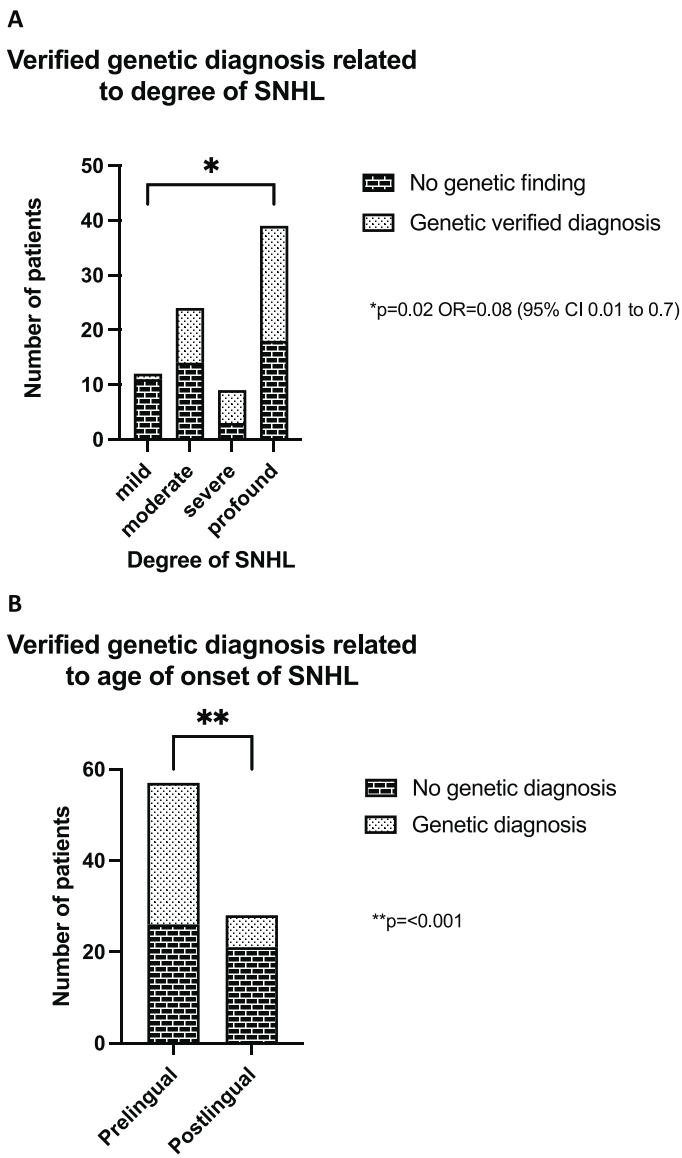

Figure 3. (A) Genetic verified diagnosis related to degree of sensorineural hearing loss (SNHL). (B) Genetic verified diagnosis related to age of onset of SNHL.

There is currently no approved gene therapy available for clinical use for patients with SNHL. Nevertheless, the first results of a clinical trial for inherited hearing loss due to PVs in *OTOF* has been published (the study is still ongoing).[31] The rapid development of such treatment is an exciting field, but it is yet unclear how efficient such therapies will be compared to treatment with hearing aids or CI. The value of genetic typing of people with SNHL is currently limited to the intrinsic value that the knowledge can offer to the family, as well as enabling specific patient-tailored follow-up. The genotype can facilitate

Table 2. Comparison of genetic yield in subgroups of sensorineural hearing loss.

(A) Comparison of Ratio of Patients Receiving a Genetic Diagnosis Between SNHL Severity Sub-Groups, Calculated with Fisher´s Exact Test: Exact Sig. (2-sided)

	Mild	Moderate	Severe	Profound
Mild		$P = .6$	$P = .02$	$P = .01$
Moderate			$P = .3$	$P = .5$
Severe				$P = .5$

(B) Logistic Regression Analyzes of Predictors Affecting Odds of Patients with SNHL Receiving a Genetic Diagnosis. There is an increased OR in receiving genetic diagnosis in prelingual SNHL in both the unadjusted and adjusted model

	Unadjusted, OR (95% CI)	Adjusted, OR (95% CI)
Severity of hearing loss		
Mild	0.1 (0.01-0.7)	0.1 (0.01-1.1)
Moderate	0.6 (0.2-1.8)	0.8 (0.3-2.6)
Severe	1.8 (0.4-8.3)	3.4 (0.6-19.8)
Profound (ref)		
Time of onset		
Prelingual	6.3 (2.1-19.0)	6.6 (1.9-22.6)
Postlingual (ref)		

the identification of symptoms and signs from other organ systems involved in a syndromic disease. While we recognize that mild hearing loss affects language development and may cause communication problems, we believe that there is a rationale to propose genetic testing in the first instance for patients with moderate-to-profound SNHL and in particular for cases with prelingual onset, based on the expected genetic diagnostic yield.

Usher Syndrome and Related Genetic Variants Pose New Challenges for the Clinician

Usher syndrome was the most common syndromic presentation in this cohort (n = 6). Early identification of a decreased peripheral vision can be detected with ERG. Genetic testing allows for detection of PVs in genes related to Usher before the vision impairment is symptomatic. Usher syndrome is divided into 4 subtypes based on symptomatology and onset. USH1 is the most severe form with profound SNHL, vestibular dysfunction, and progressive RP from birth, while USH2 is the most common subtype with usually normal vestibular function and progressive hearing loss and visual impairment during puberty. In USH3, SNHL and impaired vision occur somewhat later in life, and USH4 is an atypical form with even later onset. We found 4 cases with USH1 and 2 with USH2, and the variants were found in the expected genes.[12]

Variants in Usher-related genes where the phenotype could either be isolated SNHL or Usher syndrome, and VUS in Usher-related genes, pose a particular challenge for the clinician, as well as for the patients and their families. Usher syndrome is clinically defined as a combination of manifest SNHL and RP. However, for USH2, the natural phenotype is normal vision until adolescence and can include vestibular dysfunction. Thus, with genetic PVs in Usher-related genes the SNHL can be diagnosed as syndromic before vision deterioration. This, and PVs in genes with several possible phenotypes, will pose new challenges to otolaryngologists and audiologists, requiring understanding of the complexities of genetics. Instead of just informing patients and caregivers about a manifest disease, with a well-described expected clinical trajectory, information has to be given with a higher level of uncertainty which from our experience may be very stressful for the families.

Consanguinity Affects the Rate of Hearing Loss in Sweden

Compared to the general population in Skåne and Örebro regions, where 30% of residents had a foreign background, 42% had foreign background in our cohort. Recently, Boudewyns et al described a Belgian cohort of 238 patients with hearing loss and a diagnostic yield of 39.5%, where around 40% were non-Europeans, mostly from North Africa and the Middle East. They found that a confirmed genetic diagnosis was more frequent in probands from North Africa (67%) and the Middle East (55%). Among the patients with a genetic diagnosis and non-European origin, consanguinity was spontaneously self-reported in almost 70% of the cases.[20] In a study from Saudi Arabia,[32] in a population with 56% consanguinity 83% of children with hereditary SNHL had related parents. Sanyelbha et al also conducted a study in Saudi Arabia and described a prevalence of SNHL of 1.4% to 1.7% in the population compared to 0.1% to 0.3% in western countries. There was an increased risk of 76% of having a child with

Table 3. Patients With Verified Genetic Diagnosis.

Patient number	Age at hearing loss diagnosis	ACMG criteria	Inheritance; variant type(s) and gene	Variants	4fPTA (right/left ear)	Isolated or syndromic SNHL	Comorbidity	Heredity, hearing loss in family and parents' country of birth
(A) Mild hearing loss (21-40 dB HL)								
1	4 y	4	AR; heterozygous in4-frame indel variant in *MYO7A*	NM_000260.4(*MYO7A*):c.4544_4551delinsCA;p.(Glu1515_Met1517delinsAla)	4fPTA 38/34	Isolated SNHL	ERG without retinal changes	1(2) younger sibling has mild SNHL (ERG not preformed) Sweden
		4	heterozygous missense variant in *MYO7A*	NM_000260.4(MYO7A):c.5494C>T;p.(Arg1832Trp)				
(B) Moderate hearing loss (41-60 dB HL)								
13	3 mo	5	AR; homozygous deletion including the *STRC* and *CATSPER2* genes	seq[GRCh38] 15q15.3(43596411_43659185)x0	4fPTA 41/46	Isolated SNHL		Sweden
14	<1 y	5	AR; homozygous deletion including exon 19–29 of *STRC*	seq[GRCh38] 15q15.3(43596411_43605610)x0	4fPTA 43/43	Isolated SNHL	Migraine	Sweden
15	3 mo	5	AR; heterozygous deletion including *STRC*	seq[GRCh38] 15q15.3(43580201_43681200)x1	4fPTA 43/45	Isolated SNHL	Autism, language disorder	Sweden
		5	heterozygous frameshift variant in *STRC*	NM_153700.2(STRC):c.2171_2174del;p.(Val724Glyfs*6)				
16	2 mo	5	AR; homozygous splice variant in *CABP2*	NM_016366.3(*CABP2*):c.637+1G>T;p.?	ABR 55/55	Isolated SNHL		Parents are cousins 1(2) sibling has SNHL Iraq
17	<1 y	5	AR; homozygous deletion including exon 19-29 of *STRC*	seq[GRCh38] 15q15.3(43596601_43605900)x0	4fPTA 38/43	Isolated SNHL	Treated with growth hormone	1(1) sibling has SNHL Sweden
18	3 mo	5	AR; heterozygous deletion including exon 22-32 of *USH2A*	seq[GRCh38] 1q41(216037606_216127696)x1	4fPTA 53/58	Usher syndrome type 2A		Sweden
		5	heterozygous splice variant in *USH2A*	NM_206933.4(USH2A):c.9371+1G>C;p.?				
19	1.5 y	4	AR; homozygous nonsense variant in *COL11A2*	NM_080680.3(*COL11A2*):c.4798C>T;p.(Arg1600*)	ABR 55/55	Stickler syndrome type III		Parents are relatives Syria
20	<1 y	4	AR; heterozygous missense variant in *SLC26A4*	NM_000441.2(SLC26A4):c.1574C>T;p.(Pro525Leu)	ABR 60/60	Pendred syndrome	Follow up by pediatrician regarding thyroid status	Lebanon
		4	heterozygous deletion encompassing exon 4-6 of *SLC26A4*	seq[GRCh38] 7q22.3(107668671_107682230)x1				
21	<1 y	5	AD; heterozygous frameshift variant in *ANKRD11*	NM_013275.6(ANKRD11):c.6031_6041del;p.(Ser2011Valfs*17)	4fPTA 40/41(air) 5/13 (bone)	KBG syndrome	Conductive hearing loss. Abnormal ossicular chain. Seizures at 8 y. Velopharyngeal insufficiency	Sweden
22	11 y	5	AD; heterozygous deletion including the *FOXC1* gene	seq[GRCh38] 6p25.3p25.2(150701_3298100)x1	4fPTA 39/49	Axenfeld-Rieger syndrome	Intellectual disability, glaucoma	Syria
(C) Severe hearing loss (61-80 dB HL)								
37	1 y	4	AR; homozygous frameshift variant i *TECTA*	NM_005422.4(*TECTA*):c.4147_4150del;p.(Val1384Alafs*10)	4fPTA 70/70	Isolated SNHL		Parents are cousins 2(3) siblings have SNHL Syria
38	1 mo	4	AR; homozygous missense variant in *MYO7A*	NM_000260.4(*MYO7A*):c.287C>T;p.(Thr96Met)	ASSR 65-70	Isolated SNHL	ERG without retinal changes	1(2) sibling has SNHL Kosovo
39	4 y	4	AD; heterozygous frameshift variant in *MYO6*	NM_004999.4(*MYO6*):c.2751del;p.(Lys917Asnfs*10)	4fPTA 64/73	Isolated SNHL		Father and 1(3) sibling has SNHL Sweden
40	1 mo	4	AR; homozygous frameshift variant in *LOXHD1*	NM_001384474.1(*LOXHD1*):c.71del;p.(Leu24Argfs*74)	ABR 70/70	Isolated SNHL		Parents are cousins 1(4) sibling has SNHL Afganistan
41	2 mo	5	AR; homozygous nonsense variant in *TRIOBP*	NM_001039141.3(*TRIOBP*):c.1039C>T;p.(Arg347*)	ABR 60/60	Isolated SNHL		Parents are cousins, one uncle deaf 1(1) sibling has SNHL Eritrea

(continued)

Table 3. (continued)

Patient number	Age at hearing loss diagnosis	ACMG criteria	Inheritance; variant type(s) and gene	Variants	4fPTA (right/left ear)	Isolated or syndromic SNHL	Comorbidity	Heredity, hearing loss in family and parents' country of birth
(D) Profound hearing loss (>80 dB HL)								
42	1 mo	5	AR; heterozygous frameshift variant in *GJB2*	NM_004004.6(GJB2):c.35del;p.(Gly12Valfs*2)	4fPTA 73/63	Isolated SNHL		Sweden
		5	heterozygous missense variant in *GJB2*	NM_004004.6(GJB2):c.94C>T;p.(Arg32Cys)				
46	2 mo	5	AR; homozygous nonsense variant in *GJB2*	NM_004004.6(*GJB2*):c.71G>A;p.(Trp24*)	ABR >90/>90	Isolated SNHL	Normal vestibular function (VHIT)	Albania
47	1.5 y	5	AR; homozygous frameshift variant in *GJB2*	NM_004004.6(*GJB2*):c.35del;p.(Gly12Valfs*2)	Deaf, diagnosed abroad	Isolated SNHL	—	Poland
48	3 mo	5	AR; homozygous nonsense variant in *GJB2*	NM_004004.6(*GJB2*):c.71G>A;p.(Trp24*)	ABR >90/80	Isolated SNHL	Congenital cholesteatoma left ear	Hearing loss within the family of the mother Macedonia
49	2 mo	5	AR; heterozygous frameshift variant in *GJB2*	NM_004004.6(GJB2):c.35del;p.(Gly12Valfs*2)	ABR >80/>80	Isolated SNHL	Down syndrome	3(6) siblings have SNHL Syria
		5	heterozygous in-frame deletion in *GJB2*	NM_004004.6(GJB2):c.358_360del;p.(Glu120del)				
50	4 mo	5	AR; homozygous frameshift variant in *GJB2*	NM_004004.6(*GJB2*):c.35del;p.(Gly12Valfs*2)	ABR >80/>80	Isolated SNHL	—	Serbia
51	1 mo	4	AR; heterozygous missense variant in *TMPRSS3*	NM_001256317.3(*TMPRSS3*):c.310G>A;p.(Glu104Lys)	ABR >80/>80	Isolated SNHL	—	Sweden
		5	heterozygous frameshift variant in *TMPRSS3*	NM_001256317.3(TMPRSS3):c.208del;p.(His70Thrfs*19)				
52	2 mo	5	AR; homozygous splice site variant in *TMC1*	NM_138691.3(TMC1):c.236+1G>A;p.?	ABR >95/>95	Isolated SNHL	Normal vestibular function (VHIT)	Parents are relatives Kurd
53	2.5 y	5	AR; homozygous frameshift variant in *TPRN*	NM_001128228.3(TPRN):c.225_235del; p.(Gly76Alafs*150)	4fPTA 94/89	Isolated SNHL		Middle east
54	<1 y	5	AR; homozygous nonsense variant i *OTOF*	NM_194248.3(OTOF):c.1422T>G;p.(Tyr474*)	4fPTA 110/91	Isolated SNHL/ auditory neuropathy-1	Visual impairment, obesity, diabetes type 2	Parents are cousins 2(5) siblings have SNHL Iraq
55	1 mo	4	AR; homozygous frameshift variant in *MARVELD2*	NM_001038603.3(MARVELD2):c.1203del; p.(Asp402Thrfs*13)	ABR >90/>90	Isolated SNHL		Parents are cousins 1(3) sibling has SNHL Syria
56	2 mo	4	AR; heterozygous nonsense variant in *MYO15A*	NM_016239.4(MYO15A):c.4612A>T;p.(Lys1538*)	ABR >90/>90	Isolated SNHL		Sweden
		4	heterozygous frameshift variant in *MYO15A*	NM_016239.4(MYO15A):c.9642_9645del; p.(Leu3215Phefs*30)				
57	1 y	4	AR; heterozygous nonsense variant in *TMPRSS3*	NM_001256317.3(TMPRSS3):c.46C>T;p.(Arg16*)	4fPTA >109/>110	Isolated SNHL		1(3) sibling has SNHL Sweden
		5	heterozygous nonsense variant in *TMPRSS3*	NM_001256317.3(TMPRSS3):c.271C>T;p.(Arg91*)				
58	1 mo	4	AR; heterozygous nonsense variant in *PJVK*	NM_001042702.5(PJVK):c.532C>T;p.(Arg178*)	ABR >90/75	Isolated SNHL	Normal vestibular function (VHIT)	Sweden
		4	heterozygous missense variant in *PJVK*	NM_001042702.5(PJVK):c.671T>G;p.(Leu224Arg)				
59	3 mo	4	AR; homozygous frameshift variant in *MYO15A*	NM_016239.4(*MYO15A*):c.10470del; p.(Leu3491Cysfs*63)	ABR >80/>80	Isolated SNHL	Normal vestibular function (VHIT, VNG, VEMP)	Turkey
60	2 mo	4	AR; homozygous deletion encompassing exon 2 of *GRXCR1*	seq[GRCh38] 4p13(42963662_42967656)x0	4fPTA 88/93	Isolated SNHL		Parents are cousins Middle east
61	2 mo	5	AD; heterozygous synonymous variant affecting splicing in *MITF*	NM_001354604.2(*MITF*):c.1230G>A;p.(Thr410=)	ABR >80/>80	Waardenburg syndrome type 2A		Sweden
62	Childhood <13 y	4	AR; heterozygous in-frame indel variant in *MYO7A*	NM_000260.4(MYO7A):c.4544_4551delinsCA; p.(Glu1515_Met1517delinsAla)	4fPTA >116/>119	Usher syndrome type 1B	Retinitis pigmentosa	Father and 1(5) sibling have SNHL Sweden
		5	heterozygous splice variant in *MYO7A*	NM_000260.4(MYO7A):c.2187+1G>A;p.?				
63	2 mo	5	AR; homozygous nonsense variant in *USH2A*	NM_206933.4(*USH2A*):c.2610C>A;p.(Cys870*)	ABR 80/75	Usher syndrome type 2A	Normal vestibular function (VHIT)	Parents are cousins Turkey
64	1 y	3	AR; homozygous missense VUS in *MYO7A*	NM_000260.4(*MYO7A*):c.1400G>C;p.(Arg467Pro)	ABR >90/>90	Usher syndrome type 1B	Retinitis pigmentosa Bilateral vestibular dysfunction.	Palestine

(continued)

Table 3. (continued)

Patient number	Age at hearing loss diagnosis	ACMG criteria	Inheritance; variant type(s) and gene	Variants	4fPTA (right/left ear)	Isolated or syndromic SNHL	Comorbidity	Heredity, hearing loss in family and parents' country of birth
(D) Profound hearing loss (>80 dB HL)								
65	2.5 mo	5	AR; heterozygous splice variant in *USH1C*	NM_153676.4(USH1C):c.104+1G>A;p.?	ABR >80/>80	Usher syndrome type 1C		Sweden
		5	heterozygous frameshift variant in *USH1C*	NM_153676.4(USH1C):c.238dup;p.(Arg80Profs*69)				
66	<1 y	4	AR; homozygous frameshift variant in *CDH23*	NM_022124.6(*CDH23*):c.494dup;p.(Ser166Glnfs*16)	4fPTA >115/>115	Usher syndrome type 1D		Iraq
67	3 mo	5	AR; homozygous missense variant in *SLC26A4*	NM_000441.2(*SLC26A4*):c.1588T>C;p.(Tyr530His)	ABR >90/>90	Pendred syndrome	Mondini malformation, LVAS and large endolymphatic sac	Sweden

Abbreviations: Autosomal Recessive (AR), Autosomal Dominant (AD), Electroretinography (ERG), the American College of Medical Genetics and Genomics (ACMG), Four-Frequency Pure-Tone Average(4fPTA), Auditory Brainstem Response (ABR), Video Head Impulse test (VHIT), Videonystagmography (VNG), Vestibular Evoked Myogenic Potentials (VEMP), Large vestibular aqueduct syndrome (LVAS).
Waardenburg syndrome: Varying degrees of hearing loss and abnormalities in pigmentation of hair, skin and eyes.
Usher syndrome: A combination of sensorineural hearing loss (SNHL), progressive visual loss due to retinitis pigmentosa and frequently vestibular dysfunction (subgroups defined in the text).
Stickler syndrome: Systemic connective tissue disorder often associated with SNHL.
Pendred syndrome: A syndrome associated with thyroid goiterand inner ear abnormalities: SNHL, vestibular aqueduct enlargement, cochlear hypoplasia.
KBG syndrome: A syndrome associated with short stature, facial and skeletal anomalies, intellectual disability and macrodontia.
Axenfeld-Rieger syndrome: SNHL combined with ocular, dental, facial, and abdominal abnormalities.

SNHL in consanguineous marriages.[33] Also in this study, self-reported consanguinity was associated with SNHL with a homozygous AR inheritance.

The Genetics of Hearing Loss is Relatively Consistent Between Populations

In a cohort from Japan with 1120 cases of nonsyndromic hearing loss,[2] variants were seen in the same genes (except *PJVK*, *MITF*, and the 2 CNVs) as in our study. In an American cohort of nonsyndromic SNHL, genetic findings were made in 440/1119 patients (39%),[6] using the gene panel OtoSCOPE® v.5 (University of Iowa, USA). The genes, where PVs were more prevalent, were similar to this present study. Recent publications examining European populations in Germany[9] and Belgium[20] showed similarity with our study, with multiple genetic findings in *GJB2*, *MYO15A*, *TMPRSS3*, and *SLC26A4*. PVs in the genes *STRC*, *CDH23*, *TMPRSS3*, *SLC26A4*, *GJB2*, *MYO7A*, *MYO15A*, *MITF*, and *MARVELD2*, are found in all 3 studies, while variants in *PJVK*, *FOXC1*, *TRIOBP*, *GRXCR1*, *COL11A2*, *USH1C*, and *ANKRD11* are only present in the Swedish cohort. The prevalence of *GJB2* in our cohort was 7% (n = 6/85) and regarded as less than expected,[34,35] but it is comparable to recent studies from Germany (8%, n = 19/305)[9] and Belgium (8.4%, n = 20/239).[20]

Although there is considerable heterogeneity among the genetic variants leading to hearing loss, the majority of the genes involved in SNHL seem to be consistent between population groups. In this study, there were 5 recurrent variants, 1 nonsense, and 1 frameshift variant in *GJB2*, 1 indel variant in *MYO7A*, 1 deletion of *STRC* and *CATSPER*, and 1 deletion of *STRC*. The most common *GJB2* variant (n = 4) was identified in 1 patient with parents born in Sweden, Serbia, Syria, and Poland, respectively (Supplemental Table 2). Thus, the recurrent variants were relatively few, making it difficult to draw any major conclusions about how the variants segregate in different populations. There was still a substantial group of unsolved cases in this study and as a next step, a trio analysis, using parental samples as controls to enable analysis of all protein-coding genes, would likely be of value.

Conclusion

In this Swedish cohort, PVs were found across 24 different genes and the total diagnostic yield was 45%. A genetic cause was found in a majority of cases in patients with prelingual, moderate-to-profound SNHL. Early onset of SNHL favored a higher diagnostic yield. In children, the genetic diagnosis provided guidance for further investigation, especially when syndromic SNHL was suspected or identified.

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Supplemental Material

Additional supporting information is available in the online version of the article.

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Paper IV

Parental experience of whole genome sequencing for children with sensorineural hearing loss

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Abstract

This in-depth interview study explores how parents of children with sensorineural hearing loss experienced genetic testing. In addition, the experienced risks and benefits were explored.

Sensorineural hearing loss is the most common sensory impairment in children and in most cases has a genetic origin. With the help of genetic sequencing, the etiology can be identified. However, a genetic test does not influence treatment, and it is unclear whether these genetic tests are perceived as valuable by patients and parents. In this study, ten parents of genetically tested children with SNHL were interviewed and the content was analysed using inductive thematic analysis method. Three global themes were identified. In the first theme, [1)]*Limited knowledge creates uncertainty*, parents described uncertainty related to the information provided, the test result itself and to factors related to the child. The second theme, [2)]*Genetic knowledge is considered important for the family and the future*, explored the importance of knowledge. Parents wanted an explanation of what the testing meant for them to make the future predictable, and the test also had practical implications of the test. In the last category, [3)]*Knowledge adds complexity and can be challenging*, ethical considerations and risks associated with knowledge were highlighted. A genetic diagnosis can cause concern and affect family planning. The main conclusion was that parents experienced that genetic testing provided valuable information on a personal level and had practical implications.

Keywords: Sensorineural hearing loss, genetic sequencing, parental experiences, thematic analysis

Background

The last decades, genome sequencing has become available for genetic diagnostics of patients with sensorineural hearing loss (SNHL). Within the HearSeq project, DNA of 96 patients was sequenced (11 with whole exome sequencing (WES) and 85 with whole genome sequencing (WGS))(Elander et al., 2022)(Elander et al., 2025). A panel with 178-210 genes(Clinical-Genomics-Lund, 2024) (the panel was updated regularly) associated with SNHL was applied.

SNHL is the most frequent sensory deficiency affecting one to two in every thousand infants(Morton & Nance, 2006). There is a detectable genetic cause in more than half of the children with prelingual SNHL (<2 yrs)(Boudewyns et al., 2023; Mitchell & Morton, 2021). In the HearSeq cohort 52 of the 85 patients examined with WGS had prelingual moderate to profound SNHL and a genetic cause was identified in 60 % (n=31) of those patients.

In cases without a definite genetic diagnosis or other defined etiology, a genetic cause still can not be excluded. Furthermore, the symptomatology of detected genetic variants is not always fully understood, and penetrance of symptoms may vary. In addition, structural variants (e.g. deletions, duplications, translocations) not related to SNHL may sometimes be detected, despite application of the SNHL-specific gene panel. Genetic variants related to isolated SNHL predominate, whereas syndromic SNHL is expected in about 30% of cases with a genetic diagnosis(Alford et al., 2014).

Persons with SNHL are treated with hearing aids (HA) or cochlear implants (CI) and additional communication devices depending on the severity of the hearing loss. Also, hearing education interventions, as well as sign language are essential. Although a clinical gene therapy trial for pathological variants in one gene (*OTOF*) has been reported as successful(Hu et al., 2024; Lv et al., 2024; Qi et al., 2024), there is currently no genetic therapy available for clinical use to treat SNHL.

From a medical point of view, there are advantages in obtaining a genetic diagnosis: both in terms of prognostic factors and for early detection of other symptoms related to hearing loss, i.e. early diagnosis of syndromic hearing loss(Korver et al., 2017; Liming et al., 2016; Mitchell & Morton, 2021; Sloan-Heggen et al., 2016). However, a genetic test does not affect treatment. This raises the question of the importance of testing and whether testing adds value for the patients and their families. Personal utility is separated from other ways of evaluating genetic tests where analytic validity, clinical validity, clinical utility (improvement of health outcome) and ethical, legal and social issues have been the main focus(Sanderson et al., 2005). Bunnik et al. (2015)(Bunnik et al., 2015) made an interesting point, by arguing that to gain personal utility, the genetic tests must contain both meaningful information and have a purpose, i.e. both clinical validity and utility need to be assessed. Personal utility is closely linked patient-centered medicine, a holistic approach embracing personal context, values and needs. An approach, which results in optimization of the clinical outcome(Ekman et al., 2011; Gluyas, 2015). In this study, the patient-centered perspective was represented by the parental experience of genetic testing.

An Australian study from 2021(Tutty et al., 2021) aimed to generate understanding of personal utility of WES in children with SNHL. It was an inductive content analysis of text from open-ended response questionnaires completed by 67 of their parents. Among other things, they concluded that the testing led to a sense of control and empowerment and was a way to avoid unpleasant surprises in the future.

In a systematic review article from 2017, including 27 studies, personal utility of genetic sequencing in general was analysed(Kohler et al., 2017). They concluded that personal utility could be identified at both a personal (affective, cognitive, and behavioral) and a social level. Based on the results of the review study, the same research team developed a Personal Utility (PrU) scale as a tool to measure the

personal benefits of genetic testing. For parents of genetically tested children, three key factors were identified: benefits for the child, affective parental benefits, and parental control(Turbitt et al., 2024).

In the literature, the focus has been on optimizing the diagnostic procedure and describing the diagnostic yield with sequencing technics. However, patient and family perceptions of genetic testing and diagnosis have become increasingly important as medicine becomes more person-centered. As caregivers, parents are the most appropriate representatives of the child, and their views are therefore important to consider. Parental experience and perceived utility of genetic sequencing, in children with diverse symptomatology, has been described in recent studies (Halley et al., 2022; Hayeems et al., 2021; Marathe et al., 2024). Yet, parental experience of genetic sequencing in relation to SNHL needs further exploration.

The aim of this in-depth interview study, using an inductive thematic approach, was to explore how parents of children with SNHL experience genetic testing and the test result. In addition, the experienced risks and benefits with genetic testing (WGS) were penetrated.

Method

This study was approved by the Swedish Ethical Review Authority (Dnr 2022-06149-02 and 2018/282) and after oral and written information all participating parents signed a consent form.

Study design

A constructivist approach for analyzing the parental experience of a phenomenon, genetic testing in children with SNHL, was needed. In this qualitative study, thematic analysis was suitable to identify experienced risks and benefits of genetic testing of children with SNHL. An inductive approach, where the analysis is grounded in the data itself without a preexisting theory, was used(Attride-Stirling, 2001; Kiger & Varpio, 2020; Varpio et al., 2020). The data were coded and categorized in a stepwise(Attride-Stirling, 2001; Braun & Clarke, 2006), reflexive way(Braun & Clarke, 2021) by two researchers. In addition to coding and identifying themes, the process included constructing, describing, exploring and summarizing networks and interpreting patterns(Attride-Stirling, 2001). Patterns and themes related to the research question were identified. The studied phenomenon was the parental experience of genetic testing of a child. In this study genetic testing relates to the process from pretest information and follow up after test result. The inductive approach was consistent with the aims of explore the

subjective experiences without a prior theoretical model and the conceptual framework was followed throughout the study (fig 1).

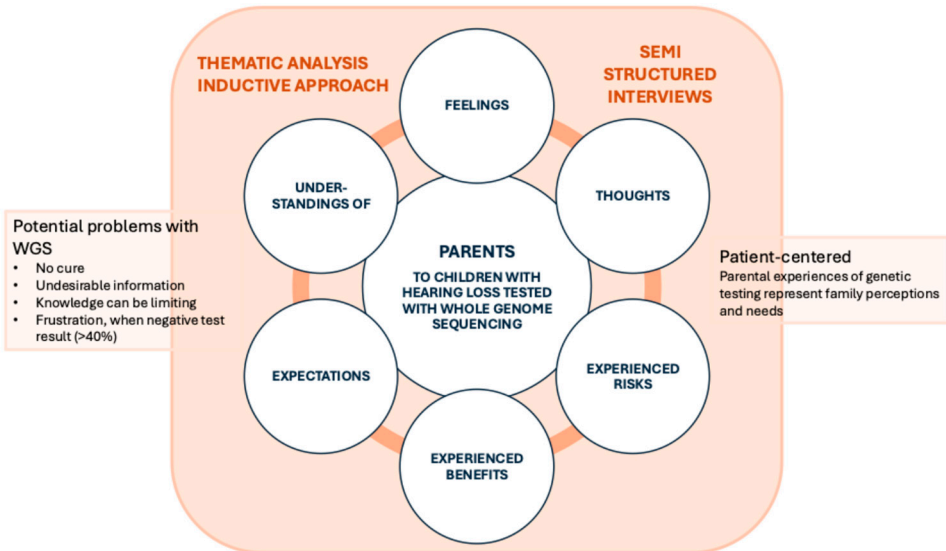

Figure 1: Conceptual framework of the study. Parents of WGS tested children with SNHL, were interviewed. The data was coded and analyzed using thematic analysis to explore how parents experienced the genetic testing.

Sampling and recruitment

The interviews were conducted in Swedish (Feb-May 2023). Ability to communicate in spoken Swedish was an inclusion criterion. Parents of the 20 genetic sequenced children, under 5 years of age, who had last undergone genetic testing at the University hospitals in Lund and Örebro, were asked by letter to participate in the study and were then contacted via a phone call. Parents of nine of the children were willing to participate in an interview and provided written informed consent (Table 1). All but one interview was made with a single parent at the time. Later, an additional, four parents were asked to participate, whereof one, not native Swedish speaker, choose to participate. In this case, Swedish was complemented with explanations in English, the second language for both the interviewer and the parent. No obvious new themes were identified. The interview was later excluded because it did not meet the inclusion criteria of being conducted in Swedish.

Table 1: Ten participating parents to children with sensorineural hearing loss (SNHL) examined with whole genome sequencing.

PARTICIPANT	SEX	DEGREE OF SNHL OF THE CHILD	GENETIC DIAGNOSIS
1	Male	Profound	None
2	Female	Moderate	None
3	Female	Mild	None
4	Female	Profound	None
5	Male/Female	Profound	Isolated SNHL
6	Female	Mild	Isolated SNHL
7	Male	Profound	Pendred syndrome
8	Female	Profound	Wardenburg syndrome
9	Female	Profound	Usher syndrome

Interview process

Semi structured interviews with ten parents (three fathers and seven mothers) were conducted by the same interviewer. An interview guide was developed by the authors JE and SW and used when conducting the interviews. The guide covered eight topics including questions about the information process, expectations, feelings, experienced risks and benefits before and after testing, and ethical considerations (supplement 1). Four of the interviews were conducted in a meeting room at the research unit in the Department of Audiology in Lund or Örebro, two were conducted in the home of the participants, while three interviews were conducted on the digital platform Zoom. All interviews were recorded. The interviewer (JE) proceeded to verbatim manual transcription of all the interviews and started the analytic process during the relistening of the interviews.

Analytic process

All the material was re-read and coded by the interviewer. Without a preexisting codebook the interviews were also read and coded by a senior researcher (SW). The codes were discussed until there was an agrement between the two researchers. The interrelated codes were clustered together and organized into basic themes. Following the inductive approach, working from the empirical data, the basic themes were organized into organizing themes. From the organizing themes, the global themes were identified, as a condensation of the concepts from the lower levels as described by Attride-Stirling (2001) and Skovdal (2015)(Attride-Stirling, 2001; M. Skovdal, 2015). This is similar to the stepwise thematic analysis process described by Clarke and Braun 2006(Braun & Clarke, 2006). They also describe potential pitfalls in the analysis process, and efforts have been made to avoid these pitfalls(Braun & Clarke, 2021). For example, the pitfall “confusing themes and topics” was identified when reviewing the themes and hence, the themes were redefined. The quotes were analyzed, and the essence of the content formed the basic themes. Sometimes quotes that at first seemed contradictory were interpreted as belonging to the same basic

theme (see example in result section 'Relief at both what was found and what was not found'). Other statements needed to be separated as they illustrated variations in reasoning. The basic themes formed the basis of the organizing themes, which were then interpreted as belonging to a global theme. The codes and themes were compared, discussed, and reassessed during the analytic process. There was a consistency in coding and a coherence in the deciphered themes. During the last interview (and the excluded interview conducted in English) no obvious new themes were generated and thus no further participants were included.

Illustration of the analytic process

Table 2 describes the analytical process for one of the three global themes, "1. Limited knowledge creates uncertainty". In the first column the organizing themes are presented, followed by the basic themes and example of the codes (quotes).

Table 2: Illustration of the first global theme

1. LIMITED KNOWLEDGE CREATES UNCERTAINTY

Organizing themes	Basic themes	Quotes
1.1 PARENTS NEED OF INFORMATION WAS NOT BEING MET	Wish for written information	*"Yes, I would need to have everything in writing."* (# 6)
	Lack of communication	A mother when receiving genetic test result about her child with Usher syndrome without any preparation. *"It was horrible. It was horrible. Because we didn't even know about it. We got a summons to the ENT specialist and that's about the only thing I can be really angry about, or not angry, but I don't know how they could have done it any better either. We got a piece of paper, and we thought we'll go to the ENT doctor and make a regular visit. We didn't know, it didn't say anything. There was nothing about, well, about genetic testing or anything in the paper."* (# 9)
	Insufficient information	*"We didn't get a lot of information really, in general, neither about syndromes nor this genetic test, but the only thing we really got was that you do a genetic test to rule out that you have a syndrome. That's what we got really"* (# 9)
	No reassurance without genetic information	A parent who wanted to take a genetic test, but the doctor hesitated and delayed the genetic testing. *"It was like this... you don't have to worry, all the time."* (# 2)
1.2 INCONCLUSIVE TEST RESULTS WERE STRESSFUL	Test result is not reliable	*"Whatever it is, if someone tells you that it's not one hundred per cent reliable, you still rely on it. Well, okay, he's got nothing, so we can rest easy, and if it turns out later that there is something, then that's where the risk lies."* (# 9)
	Vague test results cause concerns	*"It's these grey zone cases, that's it, it's hard not to get a clear answer."* (# 6)
1.3 UNCERTAINTY IF GENETIC TESTING IS IN THE BEST INTEREST OF THE CHILD	Child function and genetics do not harmonize	*"And then if you already know the genetic information about someone like that. Because it doesn't say much, it says he's deaf, but it doesn't say he has two functioning cochlear implants and sign language, signs with support and..."* (# 8)
	The test is not used in the best interest of the child	*"That it falls into the wrong hands. That's the risk, and I feel that the world is not so risk-free anymore. That you should not be completely naive. That's what it is."* (# 4)
	The child´s opinion about genetic testing is unknown	*"That is if you believe that he, we chose to find out everything about him and his genetics. Right now, as his parents. But maybe he doesn't want to know why. Maybe he just accepts that this is the way it is and doesn't want to know more."* (# 8)

Translation

For translation of the quotes from Swedish into English the AI translator DeepL (VAT-ID: DE349242045) was used, and the content of each quote was manually checked by JE and re-translated to ensure that the meaningful content was intact and unchanged.

Results

From the transcribed and coded interviews, three global themes were identified "Limited knowledge creates uncertainty", "Genetic knowledge is considered important for the family and the future" and "Genetic knowledge adds complexity and can be challenging" (fig 2). All global themes are in different ways related to knowledge. The longing for an answer and how both knowledge and not knowing influence the experience of the genetic test. Knowledge can also lead to more questions and produce outcomes apart from the primary aim of the genetic test. Three main categories were identified in each theme, which in turn in most cases were based on several sub-categories.

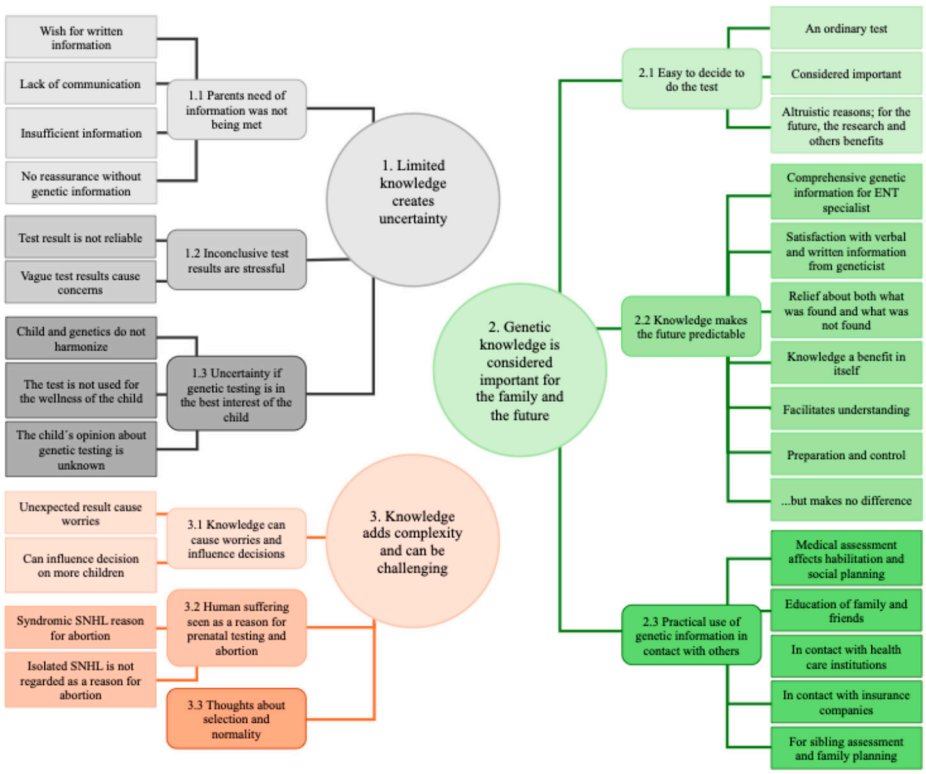

Figure 2: Three themes related to parental experience of WGS were identified "Limited knowledge creates uncertainty", "Genetic knowledge is considered important for the family and the future" and "Knowledge adds complexity and can be challenging".

Theme 1. Limited knowledge creates uncertainty

Parental experience of uncertainty centered on the information provided, the test result itself and factors related to the child.

1.1 Parents need of information was not met

Some parents experienced a gap between the parental need of information and the received information. Recognition of parental needs and expectations was crucial for the information process. There was a wish for written information, as one mother expressed as following.

> "Absolutely, you would like to have a written answer and maybe a little bit based on what we have discussed or said." (# 4)

Additionally, there were parents who felt misinformed by their treating physician mainly due to a lack of communication, both between the parent and the treating physician and between physicians with different specialist competences. Lack of communication was troublesome, exemplified in a case where the information process was disrupted by misunderstandings, long waiting times, and frustration. When doctors, with different specialties, interpreted the genetic results differently and one said that Usher syndrome was excluded, and the other ordered check-ups, the parents experienced that it was hard to know who to trust. Also, parents pointed out that they received insufficient information. Others experienced that offers from the department of clinical genetics was not being transferred to the family. This resulted in feelings of being withheld the opportunities to be properly informed about the condition of their child.

> "We have received these test results from there [clinical genetics] and it also says that if the family wishes and wants, they can be referred to clinical genetics. It says in the referral response from there. And we didn't do that, we didn't get that, that offer." (# 6)

In cases where the treating physician tried to be reassuring before the genetic test was taken or before the test result was obtained, this was experienced as counterproductive and not reassuring without genetic information. This was expressed as feelings of frustration and that their concerns were not taken seriously.

1.2 Inconclusive test results are stressful

In this second category, the limited knowledge is related to the test result itself and the built-in knowledge restriction; that there are still more to understand about genotypes related to hearing loss.

Some parents acknowledged the risk that the test was not completely reliable and that this was something that could be troublesome.

> "Well, okay, he's got nothing, so we can rest easy, and if it turns out later that there is something, then that's where the risk lies." (# 9)

Another stressful situation was to get a vague test result. For example, one child had a pathogenic variant in a gene where the phenotype could be either isolated hearing loss or Usher syndrome. This ambiguous result, and the offered eye examinations were stressful for the parents and not knowing what to expect was difficult to manage.

> "I have read quite a lot about the gene and so on and see that there is a gray area and see that it's not just black and white, it's not just either or, but that there are people with this gene who get an atypical Usher, which is a bit more like Usher type 3." (# 6)

1.3 Uncertainty if genetic testing is in the best interest of the child

In this subcategory, knowledge and uncertainties related to the child are in focus.

The genetic result might not harmonize with the functions of the child. Parents acknowledge that the genetic code can tell that someone became deaf, had visual problems or got some rare disease. However, they experienced a risk that the perception of the disease by others, may not be in line with the personality or the functional resources of the child, treated with cochlear implants, or other habilitation aids. Another potential risk identified was that the genetic data could be used for purposes other than the best interest of the child; it is not known how this will be regulated in the future. Depending on legislation and rules regulating genetic data the test could be misused, and one mother expressed it like this.

> "That it falls into the wrong hands. That's the risk, and I feel that the world is not so risk-free anymore. That you should not be completely naive." (# 4)

Also, there is an uncertainty if the child when he/she grows up favors the genetic information. Even if the guardians did what they thought was in the best interest for their child, they decided to take the test without knowing the future wishes of the child.

> " But maybe he doesn't want to know why. Maybe he just accepts that this is the way it is and doesn't want to know more." (# 8)

Theme 2: Genetic knowledge is considered important for the family and the future

This theme covers parental thoughts and opinions on the usefulness of genetic information. Knowledge tends to be the central subject. An urge after knowing why, to get an explanation of what this means for me and what it can lead to in the future

is something all the participants experienced, one way or another. The first category is linked to the decision to do the genetic test, while the subsequent categories are related to the benefits of comprehensive knowledge, for making the future predictable and practical consequences of a confirmed diagnosis.

2.1 Easy to decide to do the test

For the parents, the decision to have the child undergo a genetic test was in most cases not a difficult decision to make and it was seen as an ordinary test, a blood test.

> "For us it was more, leave sample, blood test. Prick in the arm and they are done, then we wait for a letter in the post. There was not much more." (# 7)

The genetic test was considered important and several participants said that they wanted to know why their child had SNHL.

> "We wanted to know what the cause was, and it was the least we could do, to leave some samples and see what the result is." (# 7)

Some acknowledged that the genetic test could reveal an unfavorable genetic finding. However, this was not a reason for not doing the test, rather the opposite. Parents pointed out that if the genetic test revealed any comorbidity, they wanted to have the chance to find out as much as possible to be able to be prepared for future events. Most of the parents also experienced that they wanted to contribute to knowledge in this field and be part of the research, which can be characterized as an altruistic standpoint. The genetic testing was not merely done for their own child, but also for future children and the expanding genetic mapping.

> " Even if it can't help us or our children, or... maybe it can help someone else who suffers the same in the future." (# 3)

2.2 Knowledge makes the future predictable

Knowledge is important, not merely for medical reasons, but also parents described knowledge as an opposite to uncertainty, which made the future predictable. The first prerequisite was comprehensive information. Subsequently, parents experienced that the knowledge was related to mental favorable factors, led to understanding that gave a sense of control and oft a relief. The genetic information was thus used as a tool to manage the situation of having a child with hearing loss more adequately.

Some of the parents experienced that they received comprehensive genetic information from the ENT specialist, while others expressed satisfaction with the information from the clinical geneticist. In fact, none of the participants articulated

dissatisfaction with the consultation at clinical genetics. One father expressed the following.

> "We went through, in detail, like what it's about, what symptoms you can get and what it looks like in the inner ear and etcetera, etcetera. So we got great information. We have all the information." (# 7)

An answer could also be experienced as a relief, both about what was found and what was not found. This means that even an answer without a genetic finding can be a relief. At least you find out what it is not. This may seem contradictory at first, that a genetic finding, but also a non-finding had similar outcomes. Some parents experienced relief that it was not Usher syndrome. Parents to a child, who received a genetic diagnosis confirming isolated hearing loss, where the mother had been worried that it was her fault somehow, also expressed relief.

> "It was really just a relief that had been released from the shoulders that, well, that you found out how it had happened. Instead of just walking there in your mind without knowing why it has happened." (# 5)

A father who had received a genetic result confirming a syndromic diagnosis, Pendred syndrome, felt relieved to get an answer. Although he admitted he was sad to have received the diagnosis, it helped him take measures for the future.

Furthermore, parents experienced that genetic knowledge can be beneficial in itself and is not just limited to factors that adjust or improve assessment and treatment. Parents said that they wanted to know. The knowledge seemed to be more important than the risk of receiving unwanted information.

> "I want to know, even if I get a very sad message, I still want to know" (# 9)

To get an answer facilitates understanding, and other suspected reasons for SNHL can be excluded.

> "If we had still been walking around suspecting that there was something wrong with the birth, we might have felt worse, or very bad about it." (# 7)

Parents experienced that by understanding the genetic condition they were able to explain to their children why they had a hearing loss and thus facilitating the understanding of the child as well. This was an issue also recognized by a mother with a negative genetic test.

> "It would have felt good to be able to tell my child. Explain to her why she has it, so that she had answers. It would have been easier to understand it" (# 3)

A positive genetic test result provided an opportunity for mental preparation, which led to a sense of control.

> "We think it's comforting to know like what we can expect, what problems X might have in the future, what can we help him with" (# 8)

Also, parents pointed out that the genetic information can be used as preparation. Even if the parents know that the genetic information do not affect treatment, they favor genetic information to be able to be prepared.

> "It doesn't really change anything. You love your child anyway, it's more because you want to be prepared" (# 2)

2.3 Practical use of genetic information in contact with others

In addition, the participants in the study described that genetic knowledge had practical implications and was considered important as a guide for habilitation and social and family planning. It was also used as an educational tool in social interactions with family and friends, as well as in contact with health care or other institutions.

Knowledge of associated morbidity was considered important, as was the prognosis of the hearing loss. Medical assessment affected habilitation and social planning, e.g., the need for special education or the need for additional communication skills, such as sign language.

> "I mean just such a simple thing like we live in Örebro county; we have Sweden's largest School for the deaf and blind. Imagine if we had planned to move away from here and then we find out when he is ten years old that he is starting to go blind." (# 9)

A definite genetic diagnosis can also be an educational aid in social interactions and in managing speculation among family and friends.

> "Now I am talking about close family and friends of ours who, when we told them that our daughter has hearing loss, many of them started floating away in their thoughts and reading on the internet without knowing anything. But getting this answer has helped us a lot and put some stop to the speculation going on around us. Because it hasn't been easy to hear others speculate about our child." (# 7)

Knowledge can be used in contact with health care institutions and to educate health professionals. One participant described how they always ended up with an ENT specialist when they went to the emergency room, even if they went for diarrhea and fever, only because the child had cochlear implants. The genetic knowledge of the condition of the child, Waardenburg syndrome, and what it entails, helped the parents to guide the health professionals when searching health care for other

reasons. The same family also had difficulty switching child insurance. In this case, they were able to use the genetic knowledge in contact with the insurance company, to claim that there was no other medical concerns in their child other than SNHL.

The last identified practical beneficial factor was related to sibling assessment and family planning, the usefulness of knowledge of inheritance patterns. One mother with a child with a *de novo* variant expressed it like this…

> "Because it affected, well, whether you would have more children or not. So that also became a thing. We [the parents] also got to be part of the result. Even though we originally did it for X's sake, we also got something out of it." (# 8)

Theme 3: Knowledge adds complexity and can be challenging

Risks connected to the knowledge and related ethical considerations were acknowledged by the parents. They realized that a genetic test result could give rise to worries about the child and affect family planning. Furthermore, genetic knowledge raised ethical concerns. The parents expressed that there is relation between the severity of a condition, and the relevance of prenatal diagnostics and abortion.

3.1 Knowledge can cause worries and influence decisions

Parents experienced that an unexpected result caused worries and was hard to manage. One mother expressed that even though she was well informed about possible genetic findings, it was terrible to get the unexpected Usher diagnosis.

On the other hand, parents also identified the there is a risk of worrying in advance or unnecessarily.

> "I can imagine that there may be things that you might not want to know if it is not possible to do anything about it. If you have a greater risk of getting certain diseases or so, it may not always be fun to know. However, it's good if you can do something about it and detect it early. But that's the risk you take, to worry unnecessarily." (# 3)

In addition, SNHL and a genetic identified cause could influence decisions of having more children. The participants regarded the use of genetic information, for family planning and selection purposes, as ethical.

> "We learnt that it was recessive, and all our children have a 25% risk of getting both mutations. Then we felt that we shouldn't have any more children and if I had become pregnant, we would have chosen to have an abortion. (# 6)

3.2 Human suffering seen as a reason for prenatal testing and abortion

The parents experienced that SNHL is a manageable disability with hearing aids, cochlear implants and other habilitative interventions. In cases of syndromic SNHL, they assessed that human suffering for the parent, child and even society could be a reason to refrain from having children.

> "Let's take that as an example, if I had known that, and say she had Usher syndrome, I would not have had her. I can say that one hundred per cent." (# 1)

The participants described that syndromic SNHL with multiple disabilities is a reason for abortion, not only for the sake of the family, but also for the unborn child. They expressed that a life with multiple disabilities might not be the life you wish for someone. One parent explained that testing and abortion is not only for the sake of the child but also for the benefit of society.

> "I mean, because it's a burden, even though X is our child, we love him more than anything, but it's still a burden on both the healthcare system and his future as well." (# 9)

When it came to isolated SNHL, this was not seen as a reason for prenatal diagnostics or abortion to any large extent.

> "I don't think we would have rejected a child if we had been told it was just an isolated hearing loss. We would never have done that." (# 6)

3.3 Thoughts about selection and normality

To do prenatal testing and make decisions about what to do with the information is considered to be personal, since the parents experience that it does not affect anyone else. Still, notions of normality are actualized when genetic tests are carried out and parents could see the risk that traits are deselected or seen as not wanted in the society.

> "But this is a big issue with ethics, because it involves selecting what is normal and what is not normal. And that is very difficult. Very, very difficult." (# 6)

Finaly, a mother, with twins, summoned up that it is hard to regret children who are already been born.

> "They have a great life, and they are great boys. So based on what has been when they were born and what is now, that's it. You can't opt out of that." (# 4)

Discussion

Genetic variation and pathological findings in children with SNHL have been of great interest from a medical point of view(Boudewyns et al., 2023; Downie et al., 2020; Mehta et al., 2016; Nishio & Usami, 2015; Sloan-Heggen et al., 2016; Tropitzsch et al., 2022; Zazo Seco et al., 2017) and can guide the physician to further medical assessment when syndromic hearing loss is suspected or diagnosed. However, the lack of available gene therapies raises the question of whether genetic diagnosis added value for families with children with SNHL. This interview study aimed to explore how the parents of children with SNHL experienced the genetic testing and whether they identified risks with, or benefits from, performing the test.

Main results

An important finding was that parents experienced that genetic testing provided valuable information on a personal level and had practical implications. However, it could be troublesome when the result was not clear or reliable.

The three global themes were all related to knowledge about the genetic etiology of SNHL. Limited knowledge created uncertainty, whereas knowledge was considered important for the family now and in the future. Knowledge could also add complexity and be challenging to handle. While straightforward information could make the future predictable and have positive practical implications, the opposite was stressful and could cause concerns linked to future decisions and an uncertainty about what was in the best interest for the child and the family. The genetic knowledge was considered important, and as soon as there was a definite answer the parents got a closure and were able to continue making plans for the future. On the contrary, parents with an inconclusive answer seemed to be trapped in the information process and some had difficulties to cope with uncertainty. Also, some parents may need additional information in writing or a referral to a clinical geneticist. Parents had straightforward opinions regarding decisions related to family planning and ethical considerations. Although these decisions are complex, they do not appear to be ambiguous and are likely to be based on fundamental personal values.

Result discussion

Uncertainty

Limited knowledge, by not receiving all information or not understanding the test results, as well as the inborn uncertainty of the test or child-related issues, can create uncertainty, as describe in the first theme (1 Limited knowledge creates

uncertainty). Uncertainty is related to anxiety, a response to a potential threat(Blanchard & Canteras, 2024; Grupe & Nitschke, 2013). A potential threat can be as hard to handle as a complicated medical condition. For example, Ginsburg et al. (2023) studied the impact that an inconclusive screening sequencing test for cystic fibrosis in infants had on their mothers. They concluded that mothers of children without symptoms, but with a variant of uncertain significance (VUS), suffered from anxiety and depression to the same extent as mothers of children with the disease(Ginsburg et al., 2023). Therefore, when performing genetic tests on children with SNHL, uncertainty must be reduced and managed at an individual level. It is impossible to know in advance how parents will react to the results, and which parents will have difficulties comprehending the results. Information to the parents can be provided in writing and when needed, complemented with information by a clinical geneticist. In this way, the uncertainties identified in the first category "Parents need of information was not being met (1.1)" can be handled. This is probably most important in cases with inborn uncertainties related to the test result. In an era of expanding knowledge in the field of genetics, finding VUS or unexpected pathological findings will continue. The inborn risk of uncertainty with genetic testing, is something that needs to be discussed with the parents beforehand. To reduce uncertain test results, an argument is to refrain from testing or having fewer genes in our gene panels. The problem is that without testing, the parents still are unsure or uncertain why their child has SNHL. What remains, if you do the testing, is the challenge to explain that a genetic test is not always black or white. Moreover, there may be unexpected test results where the possibility to predict the future is limited. As one interviewee pointed out, there is also uncertainty about how legislation will regulate genetic data in the future and that the data may be used for purposes other than the well-being of the tested child.

To make sure if someone want the information in writing, has an urge to get a deeper understanding about the condition or would benefit from consulting a clinical geneticist were interpreted as success factors to reduce uncertainty based on the parental experiences.

Knowledge

Our second theme can be discussed using the sense of coherence theory developed by Aaron Antonovsky more than 40 years ago(Antonovsky, 1987). He reasoned that the ability to manage difficult situations (in this case having a child with functional impairment) was connected to three elements: comprehensibility, manageability, and meaningfulness. In this context, the knowledge of why your child has SNHL provides a comprehensibility. With a known disease-causing genetic variant, speculations about other causes can be avoided. When a known variant is accompanied with the expected phenotype, the condition is understandable, and the future may become more predictable (2.2). The first category, easy to decide to do the test (2.1) is a way to reach comprehensibility, but also to create meaningfulness

by contribution to the research and other children. To use the genetic information in contact with others (2.3) is another way of using the knowledge for something meaningful.

This finding is in congruence with the Australian study(Tutty et al., 2021)about personal utility (with WES in children with SNHL) where a genetic test was related to a sense of control and empowerment. The reason for this is probably that knowledge can be anchored in reality, and the world becomes understandable. Dumez et al. argued that recognizing patient knowledge and understanding that this knowledge can be differentiated in nature is fundamental to creating value from knowledge (Dumez & L'Espérance, 2024). This broader definition of the nature of knowledge is appealing also related to this study.

Ethical considerations

The third theme is closely linked to ethical considerations. Parents identified that there is a risk of selection when introducing genetic testing usable for prenatal diagnostics and abortion. However, whether it is a true risk, or a desirable consequence of testing is a subject where there can be different opinions. The connection between genetic testing and potentially complex standpoints and decisions regarding future children did seem surprisingly uncomplicated for the parents. These may be decisions that parents have reflected on more deeply, that are part of personal values and that are therefore, despite their complexity, easy to make. Nevertheless, the feelings and thoughts about future children did not seem to affect the feelings for the present child. Whether parent-infant attachment is affected by a genetic test is sparsely mentioned in the literature. However, obtaining an early diagnosis of hearing loss/deafness (established through the newborn screening program) has been found to be appreciated by parents(Magnuson & Hergils, 1999, 2000). These experiences may be transferable to receiving an early genetic diagnosis of SNHL.

Utility and comparison with earlier studies

Utility with genetic sequencing in rare diseases can be viewed from different angles. While Hayeems et al.(2022)(Hayeems et al., 2022) studied ways to measure clinical utility, others concentrate on the patient or parental perspective of perceived utility. The themes of personal utility identified by Kohler et al.(2017)(Kohler et al., 2017) are related to the categories and subcategories identified in our study. For example, *affective personal benefits* can be identified in both category 2.1 where knowledge was considered important and in 2.2. where it was related to satisfaction and relief. *Cognitive benefits* can be considered as facilitating understanding (2.2), leading to mental preparation and control (2.2), while *behavioral benefits* can have practical implications on medical assessment, habilitation and family planning (2.3). *Social benefit* was seen in both category 2.1 (altruistic reasons, for the future, for the research and to benefit others) and in category 2.3, where it was more related to

practical consequences in social contacts. Also, the themes identified with the parental personal utility scale (parental PrU) by Turbitt and Kohler et al. (2024)(Turbitt et al., 2024): child benefits, affective parent benefits and parental control, had similarities with our results. Nevertheless, both clinical and personal utility can be regarded as important for the parents. Smith et al. (2022)(Smith et al., 2022) described five different utility domains: clinical, emotional, behavioral, cognitive and social utility. In this study too, there seems to be a congruence of themes, although with slightly different focus, interpretation and evaluation. Compared to our study, similar content is described in our categories (2, 3) with both cognitive, emotional, as well as practical benefits for both the parents and the child. However, in the themes of our study, we have focused what underlies the perceived utilities and disutility of the test. In conclusion, knowledge was considered a key factor for making informed decisions for the families.

Scientific contribution and practical implication

The scientific contribution of this study is the knowledge that parents experienced genetic knowledge as an asset, and that they wanted to understand why their child had SNHL. They experienced uncertainties as risks, but not the knowledge itself. There is a complexity in genetic investigations and therefore providing information before and after testing is essential. Many parents in the study want more information than was provided. Thus, our conclusion is that additional clinical appointments with the parents will be required, a time-consuming process in our clinical setting.

In the literature, barriers to do genetic testing in children with hearing loss have been studied. Difficulties with insurance costs and lack of genetic knowledge were highlighted from both a parental(Cejas et al., 2024) and treating physician(Heyward et al., 2023) point of view. However, where health care is financed by the government, like in Sweden, the financial limits are related to the healthcare provider and not the financial status of the parent. Given this, the clinician needs to be knowledgeable and propose testing only when it is relevant. A low probability to find a genetic pathogenic variant, where there is no available genetic treatment option or low suspicion of a syndrome, are factors that can make testing less valuable for both parents and clinician.

However, practical implication of this study is that clinicians need not hesitate or postpone the testing procedure if there is a reasonable clinical gain. Based on the results of this study, parents are generally in favor of genetic testing, and they see personal, mental and practical, gain from various perspectives and have an altruistic view of testing. On the other hand, parents that rejected testing were not included in our study. To complement the views on genetic testing for children with SNHL, it would be interesting to explore why parents refrained from testing their children.

The parental experience of the genetic investigation is, together with medical and ethical issues, important to consider when offering genetic testing to children with SNHL. Parental experience, in this field, is limited in research and this study complement the study made by Tutty et al. (2021) using open-ended questionnaires(Tutty et al., 2021). In this study, we have penetrated both the benefits and values, as well as the potential risks with testing. The outcome of this study can support physicians in deciding when and whether to offer genetic testing to children with SNHL to ensure that care is in line with what parents (and the patient) need for a good care experience.

Method discussion

Since one of the inclusion criteria was that the interviews should be in Swedish, there is a risk of cultural bias of the selected individuals. In our population, up to 40 percent of the children with SNHL have parents originating from elsewhere in the world. Only two of the participating parents in this study were born outside of Sweden. Thus, generalizations for the whole population are questionable. Nevertheless, the argument, in favor of having Swedish spoken language as an inclusion criterion, was to avoid misinterpretations. This was considered important for the reliability of the study. However, in comparison with the study by Kohler et al. (2017)(Kohler et al., 2017), where they investigated personal and social outcome with genetic testing, there are similarities in the sub categorizes between their study and ours. Their themes (affective, cognitive, behavioral and social outcomes) are quite different, compared to ours, and are more prone to be topics. However, the personal and social outcomes they describe reflect more or less all subcategories in our themes 2 and 3. As they focused on personal utility, they have not described anything corresponding to our first theme of uncertainty. Yet, the similarities in the sub-categories indicate that our findings are transferable to other settings.

As all the interviews and transcripts were made by the same person (JE); there was a consistency during this process. The interviews were considered to contain rich data, and no obvious new categories were identified during the last interviews. However, as the study contains only nine interviews, there is a chance that complementing experiences and themes could be identified with additional participants. Nevertheless, both female and male participants are represented, as well as participants from different socioeconomical backgrounds and education levels. In this context, it must also be underlined that this is a study where the parents have signed an informed consent to perform the genetic test on their child. Thus, the experience of parents who do not want the genetic test to be performed on their child has not been explored. During the interview process the material was read, coded and discussed by two researchers (JE, SW). In this sense, there was an inter-rater reliability when discussing codes and possible themes. Even though the researchers have different professions (otorhinolaryngologist and psychologist) and academic

experience (PhD student and associate professor) there was an agreement in important meaningful quotes. As both researchers are working in the field of audiology, preunderstanding and expertise could have influenced the analysis. However, this subjectivity can rather be seen as an advantage and the researchers as key instruments to grasp the content. In a reflexive manner, the initial themes were reviewed and redefined(Braun & Clarke, 2021). Once the themes were structured, the codes were re-checked and confirmed, to ensure that the meaningful units were expressed in the basic, organizing and global themes. What further increases credibility(Shenton, 2004) is the similarities of our results with the findings from Kohler et a. (2017)l(Kohler et al., 2017).

Conclusion

Based on the parental experience, the clinicians can be encouraged to offer genetic testing to children with SNHL. Not only from a medical point of view, but also from a patient-centered perspective where knowledge is considered important for the family and the future. Parents experienced that genetic testing, and genetic knowledge, was valuable on a personal level as well as for altruistic reasons. It was important for making the future predicable and had practical implications. However, to avoid anxiety the parental need of information had to be met. The risk of vague genetic test results, as well as the uncertainty related to the future wishes of the child, could not be erased. Genetic knowledge could make decisions more complex and influenced family planning and was used to make predictions about the future.

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Ethical statement

Our study was approved by the Swedish Ethical Review Authority (Dnr 2022-06149-02 and 2018/282). All participating parents received oral and written information and provided a signed consent form prior to enrollment in the study.

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Conflict of Interest

Three of the researchers are clinically involved in the care of children with hearing loss at the Department of Otorhinolaryngology in Lund and in Örebro. The researcher who conducted the interviews did not have patient responsibility for any of the children whose parents were participating in the study. The research group has no other conflicts of interest to declare.

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Genetic hearing loss in children

The genetic variation in children with hearing loss is heterogeneous and can be related to both mitochondrial and nuclear pathogenic variants. Genetic testing using whole exome or whole genome sequencing, tailored to a specific gene panel, is efficient.
In this thesis, the genetic yield in prelingual moderate to profound hearing loss cases was over fifty percent. Additionally, parents of children who underwent whole genome sequencing experienced genetic testing to be personally valuable and practically useful. However, a vague test result could cause difficulties.

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