Chapter

Auditory Neuropathy Spectrum Disorder: Genetic and Electrophysiological Testing for Predicting Rehabilitation Outcomes after Cochlear Implantation

Maria Lalayants

Abstract

Reviling the etiology or at least pathophysiology of auditory neuropathy spectrum disorder is crucial for choosing rehabilitation pathway and predicting rehabilitation outcomes. Some patients with auditory neuropathy spectrum disorder undergo cochlear implantation, but it is not always possible to foresee the results of rehabilitation. Genetic testing, especially in cases without obviously perinatal hearing loss risk factors, might help to understand etiology and pathophysiology, whether it is synaptopathy or neuropathy; therefore, it becomes possible to predict rehabilitation outcomes. More than 20 genes related to auditory neuropathy spectrum disorder phenotype are known already. Modern genetic approaches, such as whole genome and whole exome sequencing, reveal etiology of auditory neuropathy spectrum disorder in many cases. But there are still auditory neuropathy spectrum disorder cases with unknown etiology and site of the lesion. Electrophysiological methods (electrocochleography, electrically evoked brainstem potentials) might help to localize the site of lesion in hearing system and therefore help to predict rehabilitation outcomes. Electrically evoked brainstem potential testing after cochlear implantation seems to be applicable and useable tool to predict potential CI outcomes and to choose optimal rehabilitation trace.

Keywords: auditory neuropathy spectrum disorder, cochlear implantation, auditory synaptopathy, Otoferlin, genetic testing, electrically evoked brainstem potentials, eABR, ANSD

1. Introduction

Auditory neuropathy spectrum disorder (ANSD) is an electrophysiological label, that incorporates patients with hearing loss of different etiologies and pathogenesis but united based on the presence of pre-neural cochlear responses such as otoacoustic emission (OAE) and cochlear microphonics (CM) and absent or grossly abnormal auditory brain steam responses (ABRs). This electrophysiological picture might reflect

the presence of pathology of the auditory system of various etiologies in any site in the auditory system from the inter hair cells (IHC) to the brain stem (including synapses, dendrites of spiral ganglion neurons, spiral ganglion neurons themselves, the auditory nerve). Therefore, ANSD is characterized by a different course and results of rehabilitation, which in many cases are difficult to predict. Elucidating the etiology and/or patophysiology of hearing loss mechanisms in these children might help to choose an optimal rehabilitation approach and predict rehabilitation outcomes [1, 2].

2. Etiology of auditory neuropathy spectrum disorder

Despite of electrophysiological commonality of ANSD, there is a broad spectrum of audiological clinical features, which come from different possible pathophysiological mechanisms due to different etiology of ANSD. About half of the cases of ANSD in children are diagnosed in those who were born premature. ANSD risk is higher among children that were born at 32 or earlier gestational week. The mechanism of ANSD among these children is due to incomplete myelinization and affected inner hair cells, as it was demonstrated in postmortem histology -inner hair cells were affected much more than outer hair cells. Also, risk factors for the development of ANSD include hyperbilirubinemia, congenital cytomegalovirus infection, asphyxia, and low birth weight. Genetic testing of patients with ANSD during the last two and half decades revealed more than 20 genes associated with that type of hearing loss. MRI testing reviled that the electrophysiological picture of congenital ANSD might be also due to the hypoplastic cochlear nerve (cochlear nerve deficiency). ANSD might be also acquired letter in life as a result of late-onset genetic-based mechanisms or due to an autoimmune reaction [3–6].

Despite of progress and availability of modern genetic tests and imaging technologies, the etiology of many cases of congenital or acquired ANSD is still unknown.

3. Broad clinical and audiological phenotype of auditory neuropathy spectrum disorder

In addition to the two main electrophysiological features mentioned above, the clinical and audiological picture of ANSD is characterized by:

1. Thresholds of pure tone audiometry can vary from normal to profound, fluctuate, and do not correspond to ABR data.

2. Violation, first of all, of speech recognition, especially in difficult acoustic situations, tone-speech dissociation (dissociation of the degree of speech recognition impairment with the thresholds of pure tone audiometry).

3. Auditory steady-state response (ASSR) are recordable in patients with ANSD, but they do not meet both the ABR thresholds (mostly absent) and the thresholds of pure tone audiometry.

Thus, objective methods of audiological examination make it possible to establish the diagnosis of ANSD, but they do not allow to establish either the degree of hearing loss or the shape of the audiogram. As long as, in patients with ANSD, behavioral

thresholds for the perception of sounds can correspond to varying degrees of hearing loss from mild or even close to normal to profound, some patients with ANSD do not require even hearing aids, while others might require cochlear implants. At the same time, it is necessary to take into account that patients with ANSD with profound impairment of speech recognition, which does not correspond to the degree of hearing loss might become a candidate for cochlear implantation despite the degree of hearing loss. Choosing the optimal rehabilitation method is also complicated by the fluctuation of hearing thresholds in some patients, as well as the possibility of hearing improvement in others, primarily those prematurely born, with ANSD during the first 1–2 years of age. Nevertheless, many patients with ANSD undergo cochlear implantation, but it is not always possible to foresee the results of rehabilitation without understanding the pathophysiological mechanism of hearing deficit [7–9].

Pathophysiological mechanisms of ANSD according to localization of pathology in auditory pathway might be divided into two groups – auditory synaptopathy and auditory neuropathy (**Figure 1**). In the case of auditory synaptopathy, pathology is localized in inner hair cells, synapses between inner hair cells and spiral ganglion neurons (SNG) dendrites, or in dendrites itself. In these cases, we might predispose that SNG cells and cochlear nerve are preserved and electrical stimulation of the spiral ganglion through cochlear implant will compensate hearing deficit. While in the case of auditory neuropathy type of ANSD, which is due to myelinopathy or axonopathy, or hypoplastic cochlear nerve, the site of lesion is located more proximal than site of electrical stimulation through CI, therefore hearing impairment is difficult to be compensated by cochlear implant electrical stimulation [10–13].

Lack of data on the mechanism of development of ANSD in each case, with a variety of clinical and audiological manifestations of ANSD in general, is the main reason for the difficulties in classifying ANSD. At the moment there is no universally accepted, optimal classification of ANSD.

Several variants of the ANSD classification have been proposed: according to the clinical picture; localization of the pathology etcetera. For example, presynaptic and

Synaptophaty	presynaptic (OTOF, SLC17A8, CACNA1D, CABP2 genes) postsynaptic (OPA1, DIAPH3, ATP1A3 genes)
Neuropathy	Charcot-Marie-Tooth Syndrome (MPZ, PMP22, NDRG1, NFEL, GJB1, GJB3 genes), Friedreich'sAtaxia (FXN gene), Wolfram syndrome (WFS1 gene), Mohr Tranebjerg Syndrome (TIMM8A gene), COWCK Syndrome (AIFM1 gene) , Leigh syndrome (NARS2 gene)

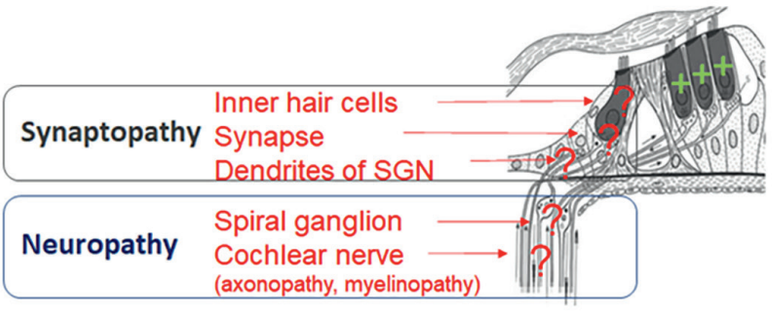

Figure 1.
Auditory synaptopathy and auditory neuropathy: Genetic cause and site of lesion.

postsynaptic. It was also proposed to highlight more detailed options: synaptopathy, ganglionopathy, myelinopathy, and axonopathy. However, classification according to such detailed morphological features, while the histological examination is impossible, can mainly be done only when a genetic mechanism is identified.

Subdivision of ANSD into synaptopathies and neuropathies seems to be the most practical. Firstly, such classification makes it possible to predict the results of cochlear implantation, and secondly, genetically determined auditory synaptopathies usually lead to the development of an isolated (nonsyndromic) hearing impairment, while genetic disorders leading to the development of neuropathy type of ANSD are most often accompanied by damage to other nerves, which might lead to syndromic form of hearing loss.

When cochlear nerve deficiency is excluded by MRI, there are two main approaches two distinguish auditory synaptopathy and neuropathy: genetic testing and electrophysiological testing.

4. Genetic cause of ANSD

More than 20 genes related to ANSD phenotype are known already. Some of them cause isolated ANSD, others—syndromic ANSD. Inheritance of ANSD can be dominant, recessive, X-linked, or mitochondrial. According to the site of lesion—genetically related auditory synaptopathy or—genetically related auditory neuropathy. Synaptophaty cases might be also divided into presynaptic or postsynaptic synaptopathy.

Genetically related ANSD can be isolated or syndromic. Mutation in SLC17A8 and DIAPH3 might cause isolated ANSD, but most cases of isolated ANSD are related to the OTOF gene mutations. Syndromic ANSD is associated with peripheral neuropathy, optic atrophy and has been linked with mutations in genes leading to Friedreich's Ataxia, Charcot-Marie-Tooth Syndrome, Leber's Optic Atrophy, Mohr Tranebjerg Syndrome, and others.

The synaptopathy type of ANSD is quite often nonsyndromic due to the unique structure of the synapses between the internal hair cells (IHCs) and the peripheral processes of the spiral ganglion neurons. They differ from synapses in the central nerve system, primarily because of synaptic bodies (ribbon synapses), resembling similar structures in retinal synapses, but which are characterized by the presence of molecules unique specifically for the auditory system. Synaptic ribbon at the presynaptic membrane holds many presynaptic vesicles with a neurotransmitter. With sound stimulation, displacement of the stereocilia of the IHCs—mechanosensitive cation channels open—cations, primarily K ions, enter the IHC, which leads to depolarization of the IHC and the opening of voltage-dependent calcium channels. Molecules of Otoferlin, located near synaptic bodies, when interacting with calcium ions, maintain the rapid simultaneous exocytosis of many presynaptic vesicles with glutamate, which ensure the transmission of the temporal aspects of an acoustic stimulus. In addition to the mentioned Otoferlin, there are other unique components in the synaptic transmission in the IHC. For example, pinocytosis (reabsorption of glutamate from the synaptic cleft) in the IHC is provided by VGluT3, unlike synapses in other structures using VGluT1 or VGluT2. The calcium channel complex also contains structures that are unique only for IHC —Ca1.3 L. The uniqueness of VGluT3, Otoferlin, and other, but not all, components of synaptic transmission in the IHC, in case of corresponding gene mutation, underline isolated form of ANSD—other organs and systems are not affected.

The proximal portion of the terminal dendrites of SGN, as well as axons in the area closer to the SG, are wrapped in Schwann cells. At the entrance to the brainstem, the axons of SGN are wrapped in oligodendrocytes. Genetic impairments in the functional consistency of these structures, primarily Schwann cells, can make it difficult to conduct a nerve impulse through SGN, especially temporal characteristics of a sound stimulus, which is primarily manifested by impaired speech recognition. Schwann cells and oligodendrocytes are also found in other parts of the nervous system, therefore in these cases, auditory neuropathy is usually not isolated, it is combined with lesions in other organs and systems. Impaired myelination, for example, in various types of Charcot-Marie-Tooth syndrome, is accompanied by a slowdown in conduction along the auditory nerve, which can, in particular, lead to impaired localization of sounds, and speech recognition, especially in noise.

So, in the case of auditory synaptopathy, the uniqueness of the corresponding synapses explains the non-syndromic nature of ANSD in most cases. Localization of the site of the lesion in the cochlear organ of Corti allows to predict satisfactory results of rehabilitation by cochlear implantation (with intact SG and overlying structures). In auditory neuropathy involving SGN, myelination may be impaired due to the pathology of Schwann cells, common in the human nervous system, which, in most cases, is accompanied by the involvement of other structures of the nervous system. Violation of the myelination of the auditory nerve, dyssynchronization is often manifested by a violation of speech recognition. Localization of the pathology is such cases does not allow one to expect satisfactory results of rehabilitation after cochlear implantation.

Genetic testing especially in cases without obvious perinatal hearing loss risk factors might help to understand etiology, pathophysiology, whether it is synaptopathy or neuropathy, and therefore, it becomes possible to predict rehabilitation outcomes.

This list of ANSD-related genes in **Figure 1** is not complete yet. A modern genetic approach, such as whole genome and whole exome sequencing, reveals new gene candidates for ANSD.

4.1 Auditory synaptopathies

Genes that lead to the development of synaptopathies, can be divided to two subgroups: presynaptic synaptopathy genes (impairment at the level of IHC, glutamate exocytosis) and postsynaptic synaptopathy genes (impairment of excitation of the dendrites of SGN). The genes for presynaptic synaptopathy include OTOF, SLC17A8, CACNA1D, and CABP2. Accordingly, these patients are promising candidates for rehabilitation by cochlear implantation. Postsynaptic synaptopathy genes include OPA1, DIAPH3, and ATP1A3. Despite that, these genes cause impairment at the level of dendrites of SGN, the body and axons of SGN and their myelinization seem to be preserved and electrical stimulation through cochlear implant might lead to good outcomes of rehabilitation.

4.1.1 OTOF gene, Otoferlin protein

According to the literature, the most common hereditary cause of ANSD is mutations in the OTOF gene encoding the Otoferlin protein. The OTOF gene and Otoferlin protein were first described in 1999. Otoferlin is expressed in the inner ear and the brain of humans. This is a calcium-dependent protein, the main function of which is the exocytosis of presynaptic vesicles with the neurotransmitter glutamate into the synaptic cleft. More than 100 mutations have already been described in the OTOF

gene, 75% of which are inactivating, leading to the absence of Otoferlin and therefore impaired synaptic transmission in the inner ear. Patients with biallelic inactivating mutations in the OTOF gene are diagnosed with bilateral non-syndromic autosomal recessive severe hearing loss (DFNB9) with an electrophysiological pattern of ANSD. The rest of the mutations are non-inactivating and lead to the synthesis of a functionally defective protein. In most cases, they lead to congenital severe hearing loss, however, cases of mild HL and even temperature-sensitive HL have been described in patients with some inactivating OTOF gene mutations—hearing thresholds are within or close to normal range, but there may be some impairment of speech recognition, especially in noise. With an increase in body temperature, sometimes even by 1°C, the patient's hearing deteriorates, while when the temperature normalizes, the hearing threshold is restored. Mutations in the OTOF gene are responsible for the development of 1.4–5% of cases of congenital non-syndromic sensorineural hearing loss, according to studies in various countries [14–16].

In different countries, there is genetic heterogeneity in the prevalence of mutations in general and the mutation profile of the OTOF gene, in particular. Thus, in Spain, the Glu829Ter mutation of the OTOF gene is not only a common cause of ANSD but is also the third most common cause of non-syndromic prelingual SNHL in general—it was detected in almost 8% of cases. In a study of the contribution of OTOF mutations to the incidence of SNHL in Japan biallelic mutations in the OTOF gene were found in 1.7% of cases, while not a single patient with the Glu829Ter mutation was found. OTOF variants, including a founder variant (p.Arg1939Gln) among Koreans, account for approximately 90% of Korean prelingual ANSD cases with anatomically intact cochlear nerves. In Russia, genetic testing of 50 children with ANSD without cochlear nerve aplasia revealed biallelic mutation of the OTOF in 12 children (24% of cases). Otoacoustic emission was recordable in all children until the last examination at the age of 12 years. In one case OAE was partly preserved even in the implanted ear 10 years after CI. No improvement, or fluctuations in hearing thresholds were noted. Ten children with OTOF-related ANSD underwent cochlear implantation, including a child with mild hearing loss, but dramatically impaired speech recognition. After cochlear implantation, the action potential of the auditory nerve to electrical stimulation and electrically evoked brainstem responses were recordable in all tested cases due to preserved cochlear nerve and the auditory pathway of the brain steam. Rehabilitation outcomes in these patients are comparable to other patients with cochlear hearing loss, like GJB2-related HL. These data are consistent with other literature describing good outcomes of CI in patients with OTOF-related ANSD [7, 13, 17, 18].

The prevalence of OTOF-related ANSD and good outcomes of CI make the search for mutations in the OTOF gene the essential component of the genetic examination of patients with ANSD, especially in early childhood hearing loss. Testing for the OTOF gene is included in many protocols for the management of children with ANSD, and the OTOF gene is included in most existing MPS gene panels for hearing loss.

The other genes that are associated with presynaptic synaptopathy, were described just in several cases now.

4.1.2 SLC17A8 gene, VGLUT3 protein: Vesicular glutamate transporter 3

Mutations in the SLC17A8 gene lead to postlingual progressive, predominantly high frequency, autosomal dominant nonsyndromic HL in humans. A study on mice showed that in mice knocked out by the slc17a8 gene synaptic transmission is impaired due to a lack of glutamate exocytosis. At the same time, eABR were

recordable, which reflected intact SGN and overlying structures and the prospects for CI as a method of rehabilitation [19].

4.1.3 CACNA1D, calcium voltage-gated channel subunit alpha1D (Cav1.3) protein

The CACNA1D gene encodes the α1 subunit involved in the formation of calcium channel pores Ca1.3. The gene is expressed in hair cells, cardiomyocytes, neurons, and neuroendocrine cells, therefore mutations in the CACNA1D gene develop a syndromic form of ANSD - prelingual hearing loss with sinoatrial node dysfunction (SANND) [20, 21].

4.1.4 CABP2, calcium-binding protein 2

CABP2, Calcium-binding protein 2, is involved in calcium entry through Ca1.3 channels. Hearing impairment in patients with a mutation in the CABP2 gene is prelingual, and moderately severe, combined with Marfan-like features in the patient [22].

4.1.5 OPA1 gene, mitochondrial dynamin-related GTPase protein

According to the literature, the most common hereditary cause of postsynaptic synaptopathy type of ANSD is mutations in OPA1 gene, which encodes the Mitochondrial dynamin-related GTPase protein. This protein is located in the inner membrane of mitochondria and is involved in many processes in the cell. Mutations in the OPA1 gene can lead to the development of non-syndromic dominant optic atrophy without hearing loss (DOA) or to dominant optic atrophy with hearing loss (DOA+). Most of the known mutations causing haploinsufficiency lead to the development of non-syndromic DOA. While, missense mutations, primarily Arg445His, probably through a dominant negative effect, lead to the formation of dominant optic atrophy with hearing loss (DOA+) Hearing impairment, in this case, is usually postlingval, after the manifestation of visual impairment. Hearing loss is progressive, of varying degrees, and audiogram profile, even within the same family. Electrocochleography in patients with DOA+, as well as studies in mice, suggested that the OHC and IHC were preserved, but the dendrites of SGN were damaged. Electrically evoked cochlear nerve action potential and eABR after cochlear implantation are recordable in patients with OPA1-related ANSD, which proved that SGN and cochlear nerve were intact in these patients. The results of cochlear implantation in patients with DOA+ are satisfactory [23, 24].

4.1.6 DIAPH3, Diaphanousformin 3 protein

The function of Diaphanusformin 3, encoded by the DIAPH3 gene, is not completely clear. However, overexpression of this gene leads to damage to SGN dendrites. A study on mice also showed the possibility of involvement in the pathological process of IHC stereocilia. Hearing impairment was postlingual, and progressive, with an autosomal dominant mode of inheritance. Satisfactory results of CI have been described in several implanted patients [25].

4.1.7 ATP1A3, Alpha-3 protein

ATP1A3 encodes Alpha-3 protein catalytic subunit of the Na+/K + ATP transmembrane ion pump that provides the resting membrane potential. The ATP1A3 gene is

expressed in the peripheral processes of SGN, in the basal ganglia, the hypocampus, and the cerebellum, therefore mutations in the ATP1A3 gene can lead to the development of the syndromic ANSD—CAPOS syndrome (hearing loss, cerebellar ataxia, areflexia, optic atrophy, cavus pes). Cases with non-syndromic HL were also described.CI in patients with mutations in the ATP1A3 gene, due to the localization of the lesion at the level of peripheral processes of SGN, led to good outcomes of rehabilitation [26].

4.2 Auditory neuropathies

Mutations in genes leading to the development of polyneuropathies cause "true" auditory neuropathies with damage to spiral ganglion neurons and/or demyelination of auditory nerve fibers. Auditory neuropathy-type hearing loss has been described in some patients with mutations in mitochondrial genes: MTND4 (Leber's syndrome), TMEM126A. The largest group of patients with auditory neuropathy type of ANSD is Charcot-Marie-Tooth syndrome [10, 11, 13, 27, 28].

Charcot-Marie-Tooth syndrome (CMT), or hereditary motor sensory neuropathies, is a clinically and genetically heterogeneous group of slowly progressive hereditary neuropathies with wide phenotype variation. CMT syndrome can be inherited in an autosomal dominant, autosomal recessive, or X-linked manner. Mutations in more than 60 genes can cause CMT, but mutations in the PMP22, MPZ, GJB1, and MFN2 genes are the most common.

The clinical picture is dominated by distal muscle wasting and weakness, decreased tendon reflexes, and distal, usually symmetrical, desensitization. Patients with CMT may also have signs of damage to other nerves, leading, respectively, the visual impairments in the form of optical atrophy, atrophy of the tongue, dysfunction of the vocal cords, diaphragm, as well as hearing loss. Sensorineural hearing loss has been described in cases related to mutations in these genes: MPZ, PMP22, NEFL, SH3TC2, NDRG1, GJB1, AIFM1, PRPS1 and INF2. Considering the pathophysiology of disorders in CMT syndrome, myelinopathy and axonopathy, it can be assumed that in patients with CMT, hearing loss will be in form of auditory neuropathy. However, in most cases, the examination of patients with CMT is limited with PTA and does not include the recording of ABR, therefore, auditory neuropathy in these cases might be missed. Moreover, even in cases where the data of pure-tone audiometry are within the normal range, auditory neuropathy cannot be excluded in a patient without registration of ABR, especially since some patients have impaired speech recognition, especially in noise. Among above-listed genes associated with hearing loss in CMT the most frequent is the MPZ gene [28–30].

4.2.1 MPZ gene, Myelin protein-zero

MPZ gene encodes Myelin protein-zero and is expressed only in Schwann cells. The main type of mutation is the point. In a patient with a mutation in the MPZ gene and HL, a considerable lesion of ganglion cells and auditory nerve fibers with a preserved IHC and practically intact OHC were described. The MPZ protein plays an important but complex role in myelination. One of the functions of MPZ, which has been confirmed by many studies, is the mediation of the process of myelin compaction through the adhesion of opposing membrane structures. MPZ acts as a homophilic molecule, the extracellular portion of which, expressed on one membrane surface, can interact with a similar region of the same protein expressed on another membrane surface. Mice in which MPZ expression was inactivated had non-compacted myelin sheaths and severe peripheral neuropathy. Therefore, mutations in regions of the MPZ

responsible for protein-protein interaction cause a more severe clinical phenotype than mutations in other regions. MPZ also has a regulatory function in the myelination process. Mice in which MPZ expression has been inactivated have dysregulated myelin gene expression and malposition of several non-compacted myelin proteins.

Hearing impairment as well as pupillary abnormalities have been described in Asp75Val and Thr124Met mutations - CMT type 2 J. With Glu97Val mutation in patients with CMT 2 J. Seeman et al. (2004) noted that hearing loss may appear up to 10 years before the onset of muscle weakness. Mild hearing loss has been noted with the Pro105Thr mutation. The disease usually manifests in the 2nd or 3rd decade of life and has a progressive course. Since, in most cases, registration of ABRs was not used to examine patients, the diagnosis of auditory neuropathy could not be detected [31].

A comprehensive survey of families with the Tyr145Ser mutation in MPZ was performed by Starr A. et al. (2003). The audiological examination included registration of ABR, OAE, cortical potentials, and psychoacoustic tests, which made it possible to describe auditory neuropathy in family members with the mutation. Pathological and histological examinations revealed not only demyelination but also a decrease in the number of spiral ganglion neurons by more than 90%. At the same time, OHC and IHC were preserved (but in the apex of the cochlea, the number of OHC was reduced). In the described cases of cochlear implantation, despite the improvement in sound perception, CI does not lead to a significant improvement in speech perception, probably due to the death of SG neurons described by Arnold Starr [30].

4.2.2 PMP22

PMP22 gene encodes a 22-kD protein that comprises from 2 to 5% of peripheral nervous system myelin. It is produced primarily by Schwann cells and expressed in the compact portion of essentially all myelinated fibers in the peripheral nervous system. The main type of mutations in PMP22 are duplications (up to 3–4 copies) as a result of unequal crossing over.

Mild to moderate sensorineural hearing loss, with greater loss in the low and high frequencies, has been described in patients with various types of mutations in the PMP22 gene. In patients with a duplication, hearing loss is congenital and less prone to a progressive course. Hearing loss in patients with a deletion of the PMP22 site, leading to hereditary neuropathy with a tendency to pressure palsies, occurred in the second decade of life and a tendency to a progressive course. However, speech recognition was not as impaired as with mutations in the MPZ gene. Thus, according to Verhagen W (2005), speech audiometry in examined patients with mutations in the PMP22 gene corresponded to tone threshold audiometry [32].

CI in a patient with PMP22-related ANSD demonstrated improvements in speech discrimination scores, however, they do not achieve the results in the typical subset of patients receiving CI [33].

In patients with CMT, cochlear implantation may reconstitute synchronous neural activity by way of supraphysiological electrical stimulation, so they can get some benefit from CI, although not at the same level as patients with cochlear hearing loss.

4.2.3 Other syndromes with ANSD

ANSD type of hearing loss can be diagnosed in patients with Friedreich's syndrome (FXN gene) - mild hearing loss, mainly impaired speech intelligibility. In such patients, the effectiveness of the use of FM systems was noted [34].

Some mutations in the WFS1 gene can lead to the development of Wolfram's syndrome, with early childhood and congenital hearing loss, predominantly low-frequency, with diabetes and visual impairment, with an autosomal recessive type of inheritance. Other mutations lead to low-frequency hearing loss, which is inherited in an autosomal dominant manner [35].

Mutations in the TIMM8A gene lead to the development of Mohr-Tranimerg syndrome with prelingual hearing loss. Optic neuropathy, atony and paranoia appear in the second decade of life, with progressive neurodegeneration [36].

Mutations in the AIFM1 gene lead to COWCK syndrome - prellingual hearing loss, progressive neuromuscular and cognitive impairments, and progressive hypoplasia of the auditory nerve [37].

Leigh's syndrome with a progressive neurodegenerative lesion of the central nervous system and hearing loss develops due to mutations in the NARS2 gene [38].

Auditory neuropathy type ANSD and progressive neurodegenerotion do not allow to expect satisfactory results of cochlear implantation in patients with these syndromes.

Auditory neuropathy-type hearing loss has been described in some patients with mutations in mitochondrial genes: MTND4 (Leber's syndrome), TMEM126A [39].

Modern massively parallel sequencing based methods allows to find more and more new ANSD causative genes, which previously were not associated with ANSD. For example, recently two mutations were found in TWNK gene of our 8-year-old patient with ANSD.

Mutations in TWNK gene were described in patients with Perrault syndrome - autosome-recessive disease, which includes sensorineural hearing loss, cerebellar ataxia, motor and sensory neuropathy, ovarian dysfunction, opftalmoplegiya. But previously HL was not described as ANSD in TWNK related disorders. After CI the EABRS were not recordable in our patients, speech recognition was poor. These data and patophisiology of TWNK related disorders, which is based on axonopathy, lead to conclusion, that TWNK-related ANSD cause auditory neuropathy type of ANSD [40].

Patients with auditory neuropathy type of ANSD due to mutations described above might get some benefit from CI, but in most cases they do not achieve the level of rehabilitation outcomes of patients with auditory synaptopathy and cochlear HL. The possibility of a poor outcomes of CI should be discussed with patient and patients caregiver before CI.

5. Methods of genetic testing

First of all, genetic testing is highly indicated for patients with a family history of hearing loss. The probability of identifying the genetic nature of ANSD is significantly higher in the group of patients without perinatal risk factors for hearing loss and cochlear nerve deficiency on MRI.

Despite of prevalence of OTOF gene mutation among patients with ANSD, it does not as high as GJB2 gene mutation prevalence in congenital "cochlear" SNHL. Therefore, single gene testing is not rationale in case of ANSD.

Development of massively parallel sequencing (MPS) and creation of multigene panels, including panels on hearing loss, containing several dozens of "hearing loss genes", as well as the development and availability of the whole genome and whole exome sequencing method, is a promising direction in the genetic examination of patients with auditory neuropathy spectrum disease, which makes it possible to identify rare forms of hereditary hearing loss.

There are 4 main types of MPS based methods (**Figure 2**):
MPS panels (NGS gene panel).
Clinical exome sequencing.
Whole exome sequencing (WES).
Whole genome sequencing (WGS).

The complete genomic information within a sample or individual is known as the whole genome. Exons are the genome's protein-coding regions and are collectively known as the exome. Despite the exome's relatively small proportion of the whole genome (approximately 2%), exomes encode most known disease-related variants. Clinical Exome Sequencing is a test for identifying disease-causing DNA variants within the 1% of the genome which codes for proteins (exons) or flanks the regions which code for proteins (splice junctions). MPS gene panels are more targeted and analyze only known disease-associated genes for specific diseases. The advantages of panels over WES or WGS include lower cost, simpler analysis, and optimisation of variant detection in the included genes. Disadvantages include the inability to analyze or re-analyze genes not included on the MPS panel. Nevertheless, gene panels that include at least OTOF gene might be the first line genetic testing of patients with ANSD. Whereas WES or even better WGS may be second-line testing options.

WGS or at least WES are better options than clinical exome sequencing, because of genetic heterogeneity of ANSD and there are still a lot of genes and mutations to be reviled as a cause of ANSD.

The main advantages of Whole Genome Sequencing

- Analyze the whole genome, including coding, non-coding, and mitochondrial DNA
- Discover novel genomic variants (structural, single nucleotide, insertion-deletion, copy number)
- Identify previously unknown variants for future targeted studies
- Possibility re-analyze data when there is new information on the genetic cause of disease

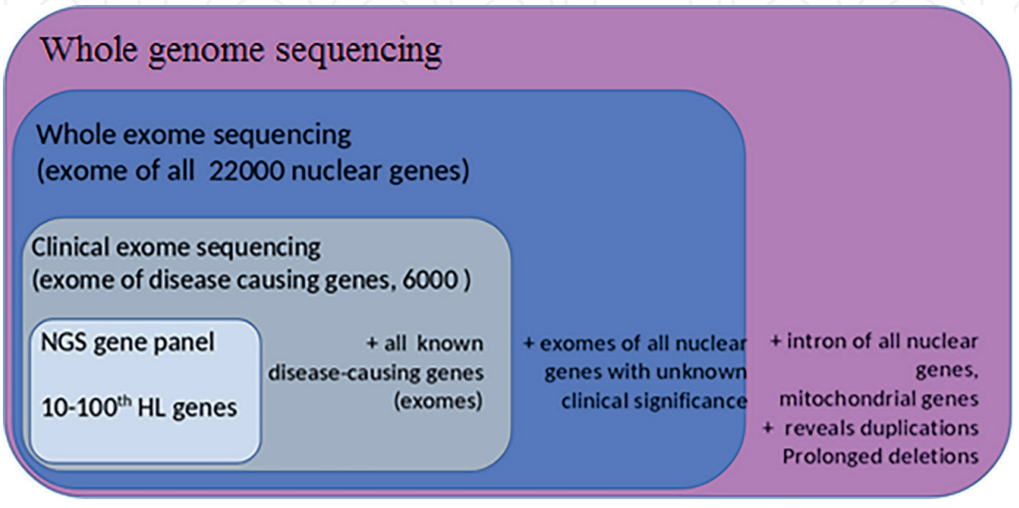

Figure 2.
Massively parallel sequencing based methods and their relationship.

The main disadvantage of WES and especially WGS is their cost and the complexity of data interpretation. Nevertheless, these methods serve an as important tool for reviling ANSD etiology in tested children and for data collection for a better understanding of ANSD mechanism in patients to come.

5.1 What if genetic testing does not revile the etiology and patophysiology of ANSD?

Even all above-described methods do not give clues in many cases. Some electrophysiological methods might help to localize the site of lesion in hearing system and therefore help to predict rehabilitation outcomes. First of all, it is electrocochleography. Presence, absence, or changes in different components of electrocochleography reflects the pathology in cochlea. Electrically evoked brainstem potentials (eABR) reflect the integrity of the hearing system up to brain steam when nerve impulse in cochlear nerve is derived by electrical stimulation. These data help to predict CI outcomes even if the etiology of hearing loss is unknown. When brainstem response to electrical stimuli is present—good CI outcomes might be expected. While eABR absence does not allow to expect good rehabilitation outcomes. Both of these methods require a stimulation electrode to be placed on the promontorium of the cochlea to achieve the clearest and reliable data. In the case of small children, these invasive procedures require sedation. If the test results indicate on electable eABR (probably synaptopathy type of ANSD), that would encourage audiologists and parents to provide cochlear implantation. But even if the test result is negative—it does not exclude cochlear implantation as an option for that child and the patient still might benefit from CI. Therefore these invasive, requiring sedation methods are not fully justified. On the other hand, EABR testing after cochlear implantation seems to be useable tool to predict CI outcomes and chose optimal rehabilitation trace after CI [41].

6. Electrically evoked auditory brainsteam responses

Electrically evoked auditory brainstem responses are bioelectrical responses from auditory pathway up to the level of the brainstem to electrical stimuli. Stimulating electrode might be situated at the promontorium, in round window night, but the largest response can be achieved when the electrode is located as close as possible to the spiral ganglion and auditory nerve. Therefore, intracochlear electrodes of CI are the best. EABR testing after CI reflects retrocochlear development after initiation of CI-mediated electrical stimulation, integrity of neural response to CI stimulation, and functional status of auditory pathway up to the brainstem. EABRs after CI can be used for verifying devise and electrode function and can assist in sound processors fitting to some extent [42, 43].

6.1 EABRs testing and morphology

EABRs might be recorded using specific for certain CI system programming software and hardware to provide stimuli (Custom SoundEP for Cochlear, SCLIN for Advanced Bionics, Maestro for Med-El) and a clinical system for evoked potentials registration. These two systems should be connected by trigger cable to synchronize stimulation and ABRs registration (**Figure 3**).

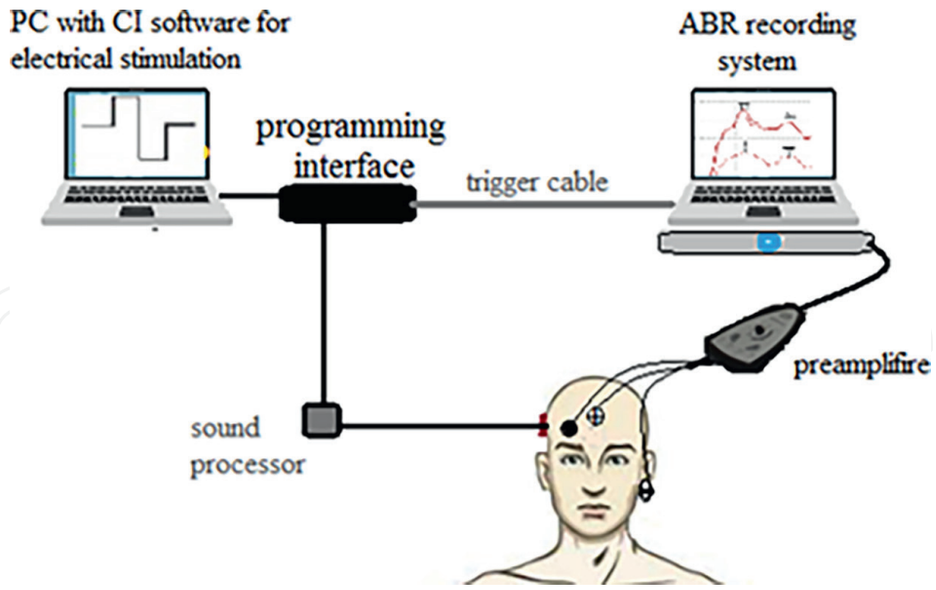

Figure 3.
EABRs recording setup.

Stimulus that is used to elict the eABRs is usually a single biphasic current pulse delivered at a repetition rate as in acoustically evoked ABR. For stimulation bipolar or monopolar electrode coupling mode might be used. Monopolar – active electrode is one of the intracochlear electrodes and indeferent electrode is extracochlear. This mode allows getting more electrode and tonotopically-specific responses. While bipolar stimulation with both intracochlear active and indeferent electrode lead to a "click"-like stimulation, which helps to elict larger response [42–45].

EABRs just as acoustically evoked ABR consist of waves II-V and usually are marked as eII-eV. Waves VI-VII usually are not seen. Wave I and sometimes wave II are consiled by stimulus artifact. There are some specific parameters and morphology of EABRs:

EABR wave latencies are 1–2 ms earlier than in ABR (but interpeak intervals are the same).

Wave V latency is approximately 4.0 ms and does not change much with an intensity of stimulation (about 0,4 ms shift between upper comfort and threshold level).

Wave V latency tends to be shorter for apical electrodes, than for basal electrodes [45].

Different parameters of eABRs were measured to asses neural response properties. First of all, eABRS thresholds. They are higher than the minimum level of electric stimulation that evokes sound sensation. This difference comes from a difference in the rate of stimulation: 25–50 stimulus per second for eABRs and 900–3000 per second for speech processor stimulation map. Therefore, eABRs, unlike ABRs, cannot be used for setting stimulation threshold levels during sound processor fitting.

Other parameters might be also explored: peak and interpeak latencies, amplitude growth function (obtained by the response amplitude, usually eV, as a function of stimulation level); channel interaction (by simultaneous stimulation of two electrode pairs); electrode positioning (lower eABR thresholds for an electrode placed near to modiolus). Waves eV and eIII latencies degrees during the first year after CI, which reflects auditory nerve and brainstem development after CI. Interestingly, there was no effect of age of CI on latency change. But despite all those parameters, the main prognostic value has eABRs present itself. The presence of eABRs is a good prognostic

sign for CI outcomes, at least it might be concluded that peripheral hearing deficiency might be compensated. This is especially important in cases of CI-challenging patients such as patients with cochlear malformation and, of course, with ANSD [45–47].

6.2 EABR testing in patients with ANSD

Several research groups during the last twenty years have studied eABR features in patients with ANSD. Some studies had showed reduced suprathreshold eV and neural dyssynchrony in patients with ANSD. The most interesting area of these research was the relationship between eABRs and speech perception after CI.

Researches compared eABRs after CI in patients with ANSD and without. It was shown that the eABR threshold and suprathreshold amplitude measures were more variable in patients with ANSD. Nevertheless, eABRs were measurable in most cases and proved that CI can provide synchronous neural responses to auditory stimulation in ANSD. EABR measures indicated that subjects with ANSD have sufficient neural sensitivity to electrical stimulation, however, they may experience less robust neural responses at suprathreshold levels. Variability in eABR parameters was most probably related to different etiology and patophysiology of ANSD.

The outcome of cochlear implantation in those patients with ANSD with recordable eABRs were not significantly different from that in other pediatric implant patients. But patients with absent eABR or with just even abnormal eABRs wave morphology had significantly worse speech outcomes. Physiologic data suggest that generally, the CI can overcome the desynchronization or whatsoever underlying ANSD and eABRs waveform are a predictor of postoperative outcomes [45–49].

EABRs were investigated in patients with ANSD with known etiology, mostly in genetically related cases.

Hosoya M. 2018 observed elongated eV wave in patients with OTOF-related ANSD. But this eV latency elongation did not affect negatively speech perception and CI outcomes. The same observation we had testing our 8 patients with OTOF-related ANSD - EABRs were recordable right after the sound processor first fitting, but even 10 years later eV latency in OTOF-related child is larger than in his pears with for example prematurity related ANSD. These data indicate that Otoferlin might play role in neurotransmission in more central part of auditory pathway than just in IHC synapses. Although there is no evidence of OTOF expression in central neural system in humans, OTOF expression was reported in rats' cerebellum [50].

EABR registration in OPA1-related ANSD revealed elongation of eV latencies. Interesting to notice that electrically evoked action potentials were absent in these patients. These finds are in concordance with the pathophysiological mechanism of OPA1-related ANSD, described above. Haploinsofiency of OPA1 protein lead to terminal dendrite dysfunction and pruning, probably therefore action potentials are absent, while spiral ganglion cells themselves are preserve, which is reflected in recordable eABRs. Patients had good speech perception after CI, as it was predicted by eABRs [24].

Despite variation in eABRs in patients with ANSD, there is no universal classification of eABRs wave morphology in patients with ANSD. Kimitaka Kaga, who was one of the first people who described ANSD in a 1994 publication, has proposed to divide eABRs in ANSD into 4 groups. Group A – pre-synaptic ANSD with normal eABR. Group B – pre-synaptic ANSD with abnormal eABR, as in OTOF-related ANSD, elongated eV latency. This elongation is attributed to secondary neurotransmission disorders due to synaptic dysfunction. Group C – EABRs are detected in post-synaptic

cases because electric stimulation could bypass the site of lesion. In this group OPA1-related ANSD and other cases of post-synaptic auditory synaptopathy cases eABRs might be included. Group D – absent eABRs in post-synaptic cases. Most demyelinating diseases and "true" auditory neuropathy cases would be included in this group.

When there is no data about the etiology of ANSD distinguishing between groups A, B, and C might be slightly difficult because eV latency might be elongated due to maturational delay in the early period after CI.

According to our experience, the main sign that electrical stimulation bypassed the site of lesion, therefore peripheral hearing deficit in ANSD patients is compensated and good CI outcomes might be expected, is the presence of eABRs (even with slightly elongated wave latency) in monopolar mode stimulation from apical, middle and basal electrodes [51].

6.3 Our experience of eABR testing in children with ANSD

Registration of eABRs was carried out in 25 children with ANSD (CI at the age of 1,5 to 5 years) at different times after cochlear implantation. 8 children were with OTOF-related ANSD, 10 with perinatal risk factors (9 prematurity with or without hyperbilirubinemia and 1 with just hyperbilirubinemia), 2 children with cochlear nerve deficiency (hypoplasia), and 5 children with unknown etiology of ANSD.

EABRS were recorded ipsilaterally, but if stimulus artifacts conceal the response, we used contralateral recordings for eABR analysis. EABRS were measured in monopolar mode, biphasic pulse stimuli with pulse duration 25–100 ms for Cochlear and 75 ms for Advanced Bionics, stimulation rate 25–26 Hz. Measurements were performed at least for three intracochlear electrodes (in basal, middle, and apical turn). If there was no response or responses in monopolar mode were inconclusive, the bipolar mode of stimulation was performed.

The standard stimulation parameters (biphasic stimulus phase width, stimulation rate) were sufficient for a clear registration of eABRs waves (eIII, eV) in all 8 children with OTOF-related ANSD and in all 10 children with perinatal risk factors for ANSD. The presence of eABRs in OTOF-related cases (**Figure 4**) matches the pathophysiology described above, and the wave latencies were elongated just as was described by other researchers [50]. Presence of eABRs in all cases with perinatal risk factors indicates that the main disorders involve inner hair cells in the case of prematurity as it was seen in histological findings [52]. Even in the case of hyperbelirubin-related ANSD, electrical stimulation bypassed the site of lesion. In children with hypoplastic cochlear nerve, EABRs were absent or partially recorded only from basal electrodes with elongated phase width up to 100 ms in monopolar mode and in case of bipolar mode of stimulation of high intensity in basal turn. In children with unknown etiology of ANSD in two cases eABRS were recordable from all stimulated electrodes in monopolar mode, in other three cases eABRs were absent or recordable only from basal electrodes at a high intensity of stimulation and/or in bipolar mode of stimulation in the basal turn of the cochlea. These patients with unknown etiology but recordable robust eABRs probably have auditory synaptopathy type of ANSD, whereas patients with absent or partially recordable eABRs have auditory neuropathy type of ANSD.

Results of rehabilitation after cochlear implantation corresponded to the results of registration of eABRs. There were much better among children with robust eABRs from all tested electrodes in monopolar mode of stimulation. All 5 children with absent or partially recordable eABRs from the basal turn in monopolar or bipolar

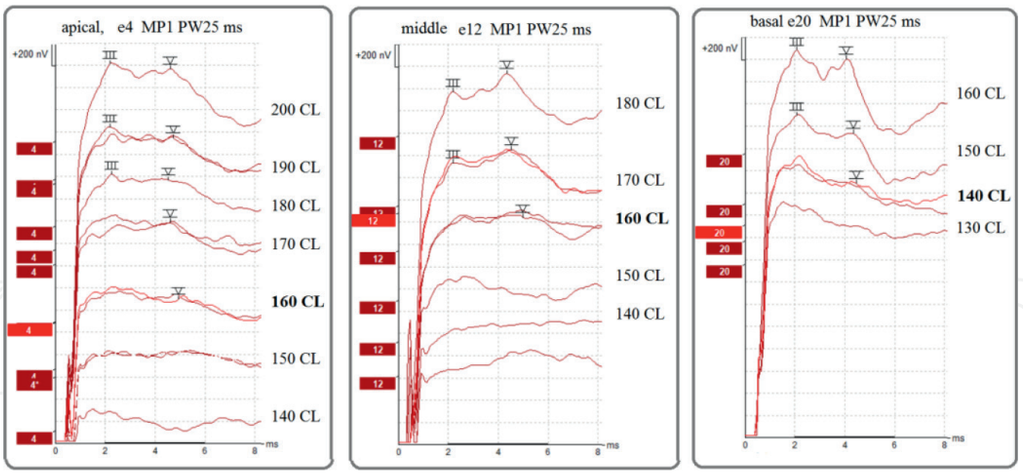

Figure 4.
EABRs from apical, middle, and basal electrodes from a child with OTOF-related ANSD, 1st day after speech processor switch-on.

mode end up with necessity of using sign language for communication. However, the selection of optimal parameters for recording eABRs made it possible to make appropriate changes to the speech processor stimulation map, which improved the perception and some discrimination of sounds. So, these 5 children had some benefit from CI, but not enough for hearing-based communication. The absence of eABRs might help to make a decision about including alternative methods of communication at an earlier stage of rehabilitation.

The thresholds of eABRs were much higher than the thresholds level of patients' speech processor stimulation map and they were closer to the maximum comfortable level of stimulation. Determination of the eABRs thresholds may help to predict the maximum comfortable level of stimulation, which is especially important in small and non-contact patients. However, this statement requires further research.

According to our data, most valuable parameters of eABRs for predicting positive outcomes after CI is recordable eABRs from intracochlear electrodes in basal, middle, and apical turn electrodes in the monopolar mode of stimulation. Latencies of eABRs do not seem to be crucial for rehabilitation outcomes. While absent or partially recordable eABRs indicate that electrical stimulation even if it causes a sound sensation, does not fully bypass the site of the lesion and cannot fully compensate hearing deficit, so an alternative way of communication might be needed.

7. Conclusions

ANSD diagnosis is not a difficult task for modern audiology, but which rehabilitation approach is optimal for the certain child and what rehabilitation outcomes we could hope for – are still quite tricky questions.

Revealing the genetic mechanism of ANSD allows understanding the lesion localization in the auditory system, which in most cases cannot be done using only standard non-invasive audiological examination methods. Identification of the genetic etiology of ANSD is extremely important, as it can make it possible to predict the course of the disease, the appearance of other symptoms in case of syndromic forms, and also to choose the optimal method of rehabilitation based on the localization of the pathology and the pathophysiology of hearing loss.

When the etiology of ANSD is unknown, registration of eABRs immediately after cochlear implantation may allow predicting the maximum rehabilitation potential of a child with ANSD. EABRs help with the choice of optimal patient management tactics, including planning the inclusion of alternative communication methods, as early as possible after cochlear implantation incase of auditory neuropathy type of ANSD.

Author details

Maria Lalayants[1,2]

1 Russian Children's Clinical Hospital of the FSAEI HE RSMU Named after N.I. Pirogov of the Ministry of Health of the Russian Federation, Moscow, Russia

2 Russian Medical Academy of Continuing Professional Education, Moscow, Russia

*Address all correspondence to: layantsmr@yandex.ru

References

[1] Kaga K, Starr A. Neuropathies of the Auditory and Vestibular Eighth Cranial Nerves. Tokyo: Springer; 2009. p. 159. DOI: 10.1007/978-4-431-09433-3

[2] Hayes D, Sininger YS. Guidelines for Identification and Management of Infants and Young Children with Auditory Neuropathy Spectrum Disorder. In: Guidlines development conference at NHS, Como, Italy. 2008. 52 p. Available from: https://www.childrenscolorado.org/globalassets/departments/ear-nose-throat/ansd-monograph.pdf on 04.03.2023

[3] Berlin CI, Hood LJ, Morlet T, Wilensky D, Li L, Mattingly KR, et al. Multi-site diagnosis and management of 260 patients with auditory neuropathy/dys-synchrony (auditory neuropathy spectrum disorder). International Journal of Audiology. 2010;**49**(1):30-43. DOI: 10.3109/14992020903160892

[4] Rajput K, Saeed M, Ahmed J, Chung M, Munro C, Patel S, et al. Findings from aetiological investigation of auditory neuropathy Spectrum disorder in children referred to cochlear implant programs. International Journal of Pediatric Otorhinolaryngology. 2019;**116**:79-83. DOI: 10.1016/j.ijporl.2018.10.010

[5] Saluja S, Agarwal A, Kler N, Amin S. Auditory neuropathy spectrum disorder in late preterm and term infants with severe jaundice. International Journal of Pediatric Otorhinolaryngology. 2010;**74**:1292-1297

[6] Saidia AR, Ruel J, Bahloul A, Chaix B, Venail F, Wang J. Current advances in gene therapies of genetic auditory neuropathy Spectrum disorder. Journal of. Clinical Medicine. 2023;**12**(3):738. [Internet] Available from:. DOI: 10.3390/jcm12030738

[7] Miyagawa M, Nishio SY, Usami SI. A comprehensive study on the etiology of patients receiving cochlear implantation with special emphasis on genetic epidemiology. Otology & Neurotology. 2016;**37**(2):e126

[8] Breneman AI, Gifford RH, Dejong MD. Cochlear implantation in children with auditory neuropathy spectrum disorder: Long-term outcomes. Journal of the American Academy of Audiology. 2012;**23**(1):5-17. DOI: 10.3766/jaaa.23.1.2

[9] Neary W, Lightfoot G. Auditory neuropathy spectrum disorder: Examples of poor progress following cochlear implantation. Audiology Medicine. 2012;**10**:142-149

[10] Shearer AE, Hansen MR. Auditory synaptopathy, auditory neuropathy, and cochlear implantation. Laryngoscope Investigated Otolaryngology. 2019;**4**(4):429-440. DOI: 10.1002/lio2.288

[11] Moser T, Starr A. Auditory neuropathy — Neural and synaptic mechanisms. Nature Reviews. Neurology. 2016;**12**:135-149. DOI: 10.1038/nrneurol.2016.10

[12] Chaudhry D, Chaudhry A, Muzaffar J, Monksfield P, Bance M. Cochlear implantation outcomes in post synaptic auditory neuropathies: A systematic review and narrative synthesis. The Journal of International Advanced Otology. 2020;**16**(3):411-431. DOI: 10.5152/iao.2020.9035

[13] Lin P-H, Wu H-P, Wu C-M, Chiang Y-T, Hsu JS, Tsai C-Y, et al. Cochlear implantation outcomes in patients with auditory neuropathy Spectrum disorder of genetic and non-genetic etiologies: A multicenter

study. Biomedicine. 2022;**10**(7):1523. [Internet] Available from:. DOI: 10.3390/biomedicines10071523

[14] Kim BJ, Jang JH, Han JH, et al. Mutational and phenotypic spectrum of OTOF-related auditory neuropathy in Koreans: Eliciting reciprocal interaction between bench and clinics. Journal of Translational Medicine. 2018;**16**:330. DOI: 10.1186/s12967-018-1708-z

[15] Rodríguez-Ballesteros M, Reynoso R, Olarte M, et al. A multicenter study on the prevalence and spectrum of mutations in the otoferlin gene (OTOF) in subjects with nonsyndromic hearing impairment and auditory neuropathy. Human Mutation. 2008;**29**(6):823-831

[16] Varga R, Avenarius MR, Kelley PM, Keats BJ, Berlin CI, Hood LJ, et al. OTOF mutations revealed by genetic analysis of hearing loss families including a potential temperature sensitive auditory neuropathy allele. Journal of Medical Genetics. 2006;**43**(7):576-581. DOI: 10.1136/jmg.2005.038612

[17] Wu CC, Hsu CJ, Huang FL, Lin YH, Lin YH, Liu TC, et al. Timing of cochlear implantation in auditory neuropathy patients with OTOF mutations: Our experience with 10 patients. Clinical Otolaryngology. 2018;**43**:352-357

[18] Santarelli R, del Castillo I, Cama E, Scimemi P, Starr A. Audibility, speech perception and processing of temporal cues in ribbon synaptic disorders due to OTOF mutations. Hearing Research. 2015;**330**(pt B):200-212

[19] Ruel J, Emery S, Nouvian R, Bersot T, Amilhon B, Van Rybroek JM, et al. Impairment of SLC17A8 encoding vesicular glutamate transporter-3, VGLUT3, underlies nonsyndromic deafness DFNA25 and inner hair cell dysfunction in null mice. American Journal of Human Genetics. 2008;**83**(2):278-292. DOI: 10.1016/j.ajhg.2008.07.008

[20] Baig SM, Koschak A, Lieb A, et al. Loss of Ca(v)1.3 (CACNA1D) function in a human channelopathy with bradycardia and congenital deafness. Nature Neuroscience. 2011;**14**(1):77-84

[21] Brandt A, Striessnig J, Moser T. CaV1.3 channels are essential for development and presynaptic activity of cochlear inner hair cells. The Journal of Neuroscience. 2003;**23**(34):10832-10840

[22] Schrauwen I, Helfmann S, Inagaki A, et al. A mutation in CABP2, expressed in cochlear hair cells, causes autosomal-recessive hearing impairment. American Journal of Human Genetics. 2012;**91**(4):636-645

[23] Huang T, Santarelli R, Starr A. Mutation of OPA1 gene causes deafness by affecting function of auditory nerve terminals. Brain Research. 2009;**1300**:97-104. DOI: 10.1016/j.brainres.2009.08.083 Epub 2009 Sep 3

[24] Santarelli R, Rossi R, Scimemi P, Cama E, Valentino ML, La Morgia C, et al. OPA1-related auditory neuropathy: Site of lesion and outcome of cochlear implantation. Brain. 2015;**138**(Pt 3):563-576. DOI: 10.1093/brain/awu378 Epub 2015 Jan 5

[25] Schoen CJ, Emery SB, Thorne MC, Ammana HR, Sliwerska E, Arnett J, et al. Increased activity of diaphanous homolog 3 (DIAPH3)/diaphanous causes hearing defects in humans with auditory neuropathy and in drosophila. Proceedings of the National Academy of Sciences of the United States of America. 2010;**107**(30):13396-13401. DOI: 10.1073/pnas.1003027107 Epub 2010 Jul 12

[26] Han K-H, Oh D-Y, Lee S, et al. ATP1A3 mutations can cause progressive

auditory neuropathy: A new gene of auditory synaptopathy. Scientific Reports. 2017;**7**(1):1-11

[27] Ćeranić B, Luxon LM. Progressive auditory neuropathy in patients with Leber's hereditary optic neuropathy. Journal of Neurology, Neurosurgery & Psychiatry. 2004;**75**:626-630. DOI: 10.1136/jnnp.2003.017673

[28] Lerat J, Magdelaine C, Roux A, Darnaud L, Beauvais-Dzugan H, Naud S, et al. Hearing loss in inherited peripheral neuropathies: Molecular diagnosis by NGS in a French series. Molecular Genetics & Genomic Medicine. 2019;**7**(9):e839. DOI: 10.1002/mgg3.839. Available from: https://onlinelibrary.wiley.com

[29] Butinar D, Starr A, Zidar J, et al. Auditory nerve is affected in one of two different point mutations of the neurofi lament light gene. Clinical Neurophysiology. 2008;**119**:367-375

[30] Starr A, Michalewski HJ, Zeng F-G, et al. Pathology and physiology of auditory neuropathy with a novel mutation in the MPZ gene (Tyr145->Ser). Brain. 2003;**126**(pt 7):1604-1619

[31] Seeman P, Mazanec R, Huehne K, Suslíková P, Keller O, Rautenstrauss B. Hearing loss as the first feature of late-onset axonal CMT disease due to a novel P0 mutation. Neurology. 2004;**63**(4):733-735. DOI: 10.1212/01.wnl.0000134605.61307.de

[32] Verhagen WI, Huygen PL, Gabreëls-Festen AA, Engelhart M, van Mierlo PJ, van Engelen BG. Sensorineural hearing impairment in patients with Pmp22 duplication, deletion, and frameshift mutations. Otology & Neurotology. 2005;**26**(3):405-414. DOI: 10.1097/01.mao.0000169769.93173.df

[33] Anzalone CL, Nuhanovic S, Olund AP, Carlson ML. Cochlear implantation in Charcot-Marie-tooth disease: Case report and review of the literature. Case Reports in Medicine. 2018;**2018**:1760978. DOI: 10.1155/2018/1760978

[34] Rance G, Corben L, Delatycki M. Auditory processing deficits in children with Friedreich ataxia. Journal of Child Neurology. 2012;**27**(9):1197-1203. DOI: 10.1177/0883073812448963

[35] Karzon R, Narayanan A, Chen L, Judith E, Lieu C. Tamara Hershey longitudinal hearing loss in Wolfram syndrome. Orphanet Journal of Rare Diseases. 2018;**13**:102. DOI: 10.1186/s13023-018-0852-0

[36] Cif L, Gonzalez V, Garcia-Ptacek S, et al. Progressive dystonia in Mohr-Tranebjaerg syndrome with cochlear implant and deep brain stimulation. Movement Disorders. 2013;**28**(6):737-738. DOI: 10.1002/mds.25519

[37] Zong L, Guan J, Ealy M, et al. Mutations in apoptosis-inducing factor cause X-linked recessive auditory neuropathy spectrum disorder. Journal of Medical Genetics. 2015;**52**(8):523-531. DOI: 10.1136/jmedgenet-2014-102961

[38] Simon M, Richard EM, Wang X, et al. Mutations of human NARS2, encoding the mitochondrial asparaginyl-tRNA synthetase, cause nonsyndromic deafness and Leigh syndrome. PLoS Genetics. 2015;**11**(3):e1005097. Published 2015 Mar 25. DOI: 10.1371/journal.pgen.1005097

[39] Feng B, Jin C, Cheng Z, Zhao X, Sun Z, Zheng X, et al. Mitochondrial dysfunction and therapeutic targets in auditory neuropathy. Neural Plasticity. 2020;**2020**:8843485. DOI: 10.1155/2020/8843485

[40] Tufatulin GS, Koroleva IV, Mefodovskaya EK, Garbaruk ES,

Levin SV, Sugarova SB, et al. Auditory neuropathy case in the child TWNK gene mutations. Innovation technology in hearing loss testing and rehabilitation of patients with hearing loss and deafness. Moscow Conference materials. 2022. p. 62-63 [in Russ]

[41] Jeon JH, Bae MR, Song MH, Noh SH, Choi KH, Choi JY. Relationship between electrically evoked auditory brainstem response and auditory performance after cochlear implant in patients with auditory neuropathy spectrum disorder. Otology & Neurotology. 2013;**34**(7):1261-1266. DOI: 10.1097/mao.0b013e318291c632

[42] User Guide for eABR Test Procedure. Eclipse with CI systems. www.Interacoustics.com

[43] Cochlear Clinical Guidance Document. 2010. www.Coclear.com

[44] Dziemba OC, Hocke T, Müller A, Kaftan H. Excitation characteristic of a bipolar stimulus for broadband stimulation in measurements of electrically evoked auditory potentials. Zeitschrift fur Medizinische Physik. 2018;**28**(1):73-77. DOI: 10.1016/j.zemedi.2017.09.008

[45] Kaga K. ABRs and electrically evoked ABRs in children. In: Modern Otology and Neurotology. Tokyo: Springer; 2022. DOI: 10.1007/978-4-431-54189-9_1

[46] Hughes ML. Objective Measures in Cochlear Implants. San Diego: Plural Pub; 2013

[47] Gordon KA, Papsin BC, Harrison RV. Activity-dependent developmental plasticity of the auditory brain stem in children who use cochlear implants. Ear and Hearing. 2003;**24**(6):485-500. DOI: 10.1097/01.aud.0000100203.65990.d4

[48] Shallop JK, Peterson A, Facer GW, Fabry LB, Driscoll CL. Cochlear implants in five cases of auditory neuropathy: Postoperative findings and progress. The Laryngoscope. 2001;**111**(4 Pt 1):555-562. DOI: 10.1097/00005537-200104000-00001

[49] Runge-Samuelson CL, Drake S, Wackym PA. Quantitative analysis of electrically evoked auditory brainstem responses in implanted children with auditory neuropathy/dyssynchrony. Otology & Neurotology. 2008;**29**(2):174-178. DOI: 10.1097/mao.0b013e31815aee4b

[50] Hosoya M. et al. Elongated EABR wave latencies observed in patients with auditory neuropathy caused by OTOF mutation //Laryngoscope Investigative Otolaryngology. – 2018. – Т. 3. – №. 5. – С. 388-393

[51] Lalayants MR, Chugunova TI, Bakhshinyan VV, Tavartkiladze GA. Electrically evoked ABR through cochlear implant in children with auditory neuropathy spectrum disorder. Vestnik Oto-Rino-Laringologii. 2022;**87**(2):4-9. DOI: 10.17116/otorino2022870214 [In Russ]

[52] Amatuzzi M, Liberman MC, Northrop C. Selective inner hair cell loss in prematurity: A temporal bone study of infants from a neonatal intensive care unit. Journal of the Association for Research in Otolaryngology. 2011;**12**(5):595-604. DOI: 10.1007/s10162-011-0273-4. Epub 2011 Jun 14