Friedreich's ataxia, and mitochondrial disease are evaluated by neurological examination to make diagnosis of syndromic AN or nonsyndromic AN. Genetic tests for appropriate genes are conducted to identify genetic cause after obtaining informed consent.

Treatment

There has been no fundamental treatment for AN. Thus, auditory rehabilitaton using hearing aids or cochlear implants plays a central role for most AN patients. However, hearing aids are not as effective in AN patients compared to non-AN patients with equivalent level of pure tone thresholds because of poor speech comprehension, which is a characteristic feature of AN. Furthermore, in general, cochlear implants have also been thought to be ineffective for AN patients because auditory neurons cannot respond correctly upon stimulation. However, this is not the case for AN caused by *OTOF* gene mutations because the auditory neurons are normal in this type of AN. Theoretically, a cochlear implant, which directly stimulates auditory neurons within the cochlea, should be effective in AN caused by *OTOF* gene mutations. In fact, successful results of cochlear implants have been reported in this type of AN [4,9]. Cochlear implant was also reported to be effective for a family with AN mapping to the AUNA1 locus.

Representative Genes Causing Nonsyndromic Auditory Neuropathy

OTOF Gene

The *OTOF* gene is the first gene identified as the cause of nonsyndromic AN. The *OTOF* gene was originally found as a locus (DFNB9: 2p22–23) that is linked to autosomal recessive, congenital, severe to profound hearing loss. Then, it was identified as a gene coding the cell membrane protein otoferlin, which is expressed in the cochlea, vestibule, and brain [10]. *OTOF* consists of 48 exons, and has multiple isoforms, by alternative splicing combined with the use of several translation initiation sites. Otoferlin belongs to a family of membrane-anchored cytosolic proteins containing six repeats of a structural module that binds calcium (the C2 domain), and they are involved in vesicle membrane fusion.

Mutant mice lacking otoferlin are profoundly deaf, with no detectable ABR across all sound frequencies tested. However, DPOAE show that outer hair cell function is maintained, as was seen in human AN patients. In these mice, the structure of the inner ear including hair cells and spiral ganglion cells is normal, but complete abolition of inner hair cell synaptic exocytosis in response to cell depolarization is detected, which is consistent with a failure of inner hair cell neurotransmitter release.

Genetic tests of *OTOF* gene were conducted in 65 American families with autosomal recessive nonsyndromic hearing loss, including 9 families with AN. Eight mutations that were related to hearing loss were found in 6 families, including 5 families with AN. One of these families, which had the I515T mutation, showed temperature-sensitive AN in which hearing loss is aggravated with elevation of body temperature and returns to mild hearing loss with normalization of the temperature. A nonsense mutation Q829X in *OTOF* gene was first identified in a Spanish population and was found in approximately 3% of autosomal recessive hearing loss in Spanish children, making it the third most frequent mutation in this population [11]. Later studies in other populations showed that the Q829X mutation also caused dysfunction of outer hair cells. Thus, it is necessary to explore the significance of this frequent mutation in both AN and non-AN sensorineural hearing loss.

Pejvakin gene

Pejvakin gene is the second gene to be identified as the cause of nonsyndromic AN [5]. This gene was identified in the DFNB59 (2q31.1-q31.3) locus by linkage analysis in two Iranian families with autosomal recessive, severe to profound, congenital hearing loss, in which T54I and R183W missense mutations were detected. Pejvakin protein consists of 352 amino acids, but its function has been unknown. Pejvakin protein is localized in the cochlear hair cells, supporting cells, spiral ganglion cells, and the first three relays of the central auditory pathway. On the other hand, dysfunction of outer hair cells was reported in a Moroccan family with insertion of T at 113–114 as well as in a Turkish family with homozygous nonsense mutation R167X and another Turkish family with homozygous missense mutation R183W which is the same mutation as in the Iranian family with non-syndromic AN. Furthermore, mutant mice that have an abnormal *pejvakin* gene demonstrated progressive hearing loss with or without the loss of otoacoustic emissions (OAE), depending on the mutation introduced in the pejvakin gene. These findings indicate that the *pejvakin* gene may cause both AN and non-AN sensorineural hearing loss, depending on the type of mutation and different background factors.

Representative Genes Causing Syndromic Auditory Neuropathy

Charcot–Marie–Tooth Disease

Charcot–Marie–Tooth disease is the most common hereditary peripheral neuropathy, characterized by slowly progressive weakness, muscle atrophy, and sensory impairment, all most marked in the distal part of the legs. Charcot–Marie–Tooth disease is classified into subtypes based on clinical features and causative genes, and hearing loss has been known to be associated with some of these

subtypes. Recently, AN was found in some of such Charcot–Marie–Tooth disease patients with hearing loss and established as a syndromic AN. The following three subtypes of Charcot–Marie–Tooth disease have been reported in association with syndromic AN.

Mutations in *PMP22* genes cause the CMT1A subtype of Charcot–Marie–Tooth disease, which shows autosomal dominant inheritance. PMP22 protein encoded by *PMP22* gene is a cell membrane protein that consists of approximately 5% of components of myelin sheath. AN has been reported in an American CMT1A family in which the A67P mutation was identified [12].

Mutations in the *MPZ* gene cause the CMT1B subtype of Charcot–Marie–Tooth disease, which shows autosomal dominant inheritance. MPZ protein coded by *MPZ* gene is a glycoprotein specific to Schwann cells, consists of approximately 50% myelin sheath components, and constitutes the myelin sheath as a complex with myelin basic protein and PMP22 protein. AN with an onset after 40 years of age has been reported in an American CMT1B family in which the Y145S mutation was identified. A study of temporal bone pathology in one member of this family revealed prominent loss of spiral ganglion cells and auditory neurons as well as well-preserved inner and outer hair cells [8].

Mutation in the *NDRG1* gene causes the CMT4D subtype of Charcot–Marie–Tooth disease, which shows autosomal recessive inheritance [13]. The *NDRG1* gene is highly expressed in Schwann cells and is expected to play a role in inhibition of mitosis and promotion of differentiation. R148X mutation in the *NDRG1* gene was identified in many European families in which AN was also found. In a CMT4D family, 25 of 39 family members complained of hearing loss that developed between 13 and 26 years of age.

Autosomal Dominant Optic Atrophy (ADOA) with Sensorineural Deafness

ADOA is a dominantly inherited disorder characterized by symmetrical optic atrophy, central visual impairment, and color vision defect. Although ADOA generally appears as an isolated disorder, it is sometimes associated with sensorineural deafness. Furthermore, some ADOA patients may be associated with not only sensorineural deafness but also several other phenotypes such as ataxia and peripheral neuropathy. Mutations in the *OPA1* gene have been found in a majority of patients with ADOA, and such mutations have also been reported in ADOA with sensorineural deafness and ADOA with deafness and other phenotypes.

The *OPA1* gene encodes a dynamin-related GTPase, which is targeted to mitochondria by an N-terminus import sequence motif and is anchored to the inner ear membrane facing the intermembrane space [14,15]. OPA1 protein is involved in the regulation of mitochondrial fusion and remodeling of mitochondrial cristae, the apoptotic process through the control of cytochrome C redistribution, and the

maintenance of mitochondrial DNA [16]. The OPA1 protein is expressed in all tissues examined, but most strongly in the retina and brain. In the ear, OPA1 protein was found to be widely expressed in the sensory and neural cochlear cells. Although the exact pathological mechanism is unknown, an abnormality of the OPA1 protein may cause an abnormality of the mitochondria, leading to insufficient energy support. This lack could then result in a dysfunction of axoplasmic transport in the nerve fibers.

In patients with ADOA and sensorineural deafness, AN was first identified in two subjects by audiological evaluation including OAE and ABR in a study of five subjects from four families having this disorder [17]. Skin fibroblasts from these subjects showed hyperfragmentation of the mitochondrial network, decreased mitochondrial membrane potential, and ATP synthesis defect, indicating that AN in these patients may be related to energy defects caused by a fragmented mitochondrial network.

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REVIEW

Value of Genetic Testing in the Otological Approach for Sensorineural Hearing Loss

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Abstract

Sensorineural hearing loss (SNHL) is one of the most common disabilities in human, and genetics is an important aspect for SNHL, especially in children. In recent 10 years, our knowledge in genetic causes of SNHL has made a significant advance, and now it is used for diagnosis and other clinical practices. Hereditary hearing loss can be classified into syndromic and nonsyndromic hearing loss. As the nonsyndromic deafness genes, more than 100 loci for deafness genes have been determined, and more than 40 genes were identified. Furthermore, more than 300 forms of syndromic hearing loss have been characterized, and each syndrome may have several causative genes. In childhood hearing loss, early educational intervention is required in addition to medical intervention for normal development of speech and language. In addition, even severe to profound hearing loss may be restored very effectively by hearing aids or cochlear implants. Because of these features of SNHL, genetic testing has exceptionally high value in the medical practice for hereditary hearing loss. Several strategies are used for genetic testing of SNHL for accurate and efficient identification of the genetic causes, and the results were used for explanation of the cause, prediction of auditory features, prevention of deafness, management of associated symptoms, determination of therapy, and genetic counseling. Identification of damaged cells in the inner ear and the underlying mechanism by genetic testing undoubtedly facilitates development and introduction of novel and specific therapies to distinct types of SNHL. (Keio J Med 58 (4) : 216–222, December 2009)

***Keywords*: hereditary hearing loss, deafness gene, inner ear, cochlea**

Introduction

Sensorineural hearing loss (SNHL) is one of the most common disabilities in human, and genetics is an important aspect in research and clinical practice for SNHL. One child in 1000 is born with bilateral SNHL, and 50-70% of them have monogenic causes.[1,2] In addition, 10% of the people over 65 years have SNHL that interfere speech communication.[3] Although most of them have polygenic causes associated with aging and various environmental causes, some of them have monogenic causes. In recent 10 years, our knowledge in monogenic causes of SNHL has made a significant advance. The knowledge of genetics in SNHL was originally established in the laboratory, but it is now used for genetic testing and following clinical procedures for patients with SNHL.

Classification of Hereditary Hearing Loss

Hereditary hearing loss can be classified into syndromic and nonsyndromic hearing loss.[4] Syndromic type which is associated with distinctive clinical features accounts for 30% of hereditary congenital hearing loss, and

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Table 1 Identified deafness genes

Autosomal dominant loci and genes			
DFNA1	DIAPH1	DFNA11	MYO7A
DFNA2	Cx31/KCNQ4	DFNA13	COL11A2
DFNA3	Cx26/Cx30	DFNA15	POU4F3
DNFA4	MYH14	DFNA17	MYH9
DFNA5	DFNA5	DFNA20/26	ACTG1
DFNA6/14	WFS1	DFNA22	MYO6
DFNA8/12	TECTA	DFNA28	TFCP2L3
DFNA9	COCH	DFNA36	TMC1
DFNA10	EYA4	DFNA48	MYO1A
Autosomal recessive loci and genes			
DFNB1	Cx26/Cx30	DFNB21	TECTA
DFNB2	MYO7A	DFNB22	OTOA
DFNB3	MYO15	DFNB23	PCDH15
DFNB4	SLC26A4	DFNB28	TRIOBP
DFNB6	TMIE	DFNB29	CLDN14
DFNB7/11	TMC1	DFNB30	MYO3A
DFNB8/10	TMPRSS3	DFNB31	WHRN
DFNB9	OTOF	DFNB36	ESPN
DFNB12	CDH23	DFNB37	MYO6
DFNB16	STRC	DFNB67	TMHS
DFNB18	USH1C		
X-linked loci and genes		Mitochondrial genes	
DFN3	POU3F4	12S rRNA	
		tRNASer(UCN)	

nonsyndromic type which is not associated with other clinical features accounts for the other 70%. Nonsyndromic hearing loss can be classified into 4 groups by the inheritance pattern, and relatively common clinical features have been noted for each inheritance pattern with a few exceptional genes, genotypes, and patients. Patients with autosomal dominant inheritance typically show progressive SNHL which begins in age 10-40, and the degree of hearing loss is various while patients with autosomal recessive inheritance most frequently show congenital and severe hearing loss. Patients with mitochondrial inheritance tend to develop progressive SNHL which begins in age 5-50, and the degree of hearing loss is various. Autosomal recessive inheritance accounts for 80% of congenital nonsyndromic hereditary hearing loss, and autosomal dominant inheritance accounts for most of the other 20%. X-linked and mitochondrial inheritance accounts for only 1-2%. After aging, the prevalence of autosomal dominant inheritance and mitochondrial inheritance increases while that of autosomal recessive inheritance decreases. The precise prevalence of each inheritance pattern is not known for adults because of the difficulty in sampling and excluding the effect of age-related hearing loss.

Deafness Genes

The first nonsyndromic deafness gene was discovered in 1993.[5] Since then, more than 100 loci for deafness genes have been determined, and more than 40 genes were identified (**Table 1**). Most of these genes play their roles within the cochlea. Thus, hereditary hearing loss almost exclusively features cochlear dysfunction.[1,2]

Although many genes are known for nonsynromic hearing loss, only a few genes including GJB2, GJB6, SLC26A4 accounts for over one third of patients with congenital hearing loss. Mutations in GJB2 account for 50% of patients with autosomal recessive hearing loss, i.e. 20% of all congenital hearing loss.[6,7] GJB2 encodes connexin 26, a gap junction protein expressed in the cochlea. Gap junctions are intercellular channels allowing recycling of potassium ions from hair cells to the stria vascularis in the cochlea and maintains a high endocochlear potential which is of critical importance for normal hearing. Mutations in GJB2 show considerable phenotypic variation, but genotype-phenotype studies showed that it is possible to predict the hearing loss associated with GJB2 mutations based on the specific genotype.[8] Combination of mutations in GJB2 and closely linked GJB6, in digenic transmission, accounts for about 8 % of deaf patients with GJB2.[9] GJB6 is a gene with sequence similarity to GJB2, is also expressed in the cochlea, and its product, connexin 30, can form gap junction with connexin 26, explaining digenic transmission of GJB2 and GJB6.

With regard to syndromic hearing loss, more than 300 forms have been characterized. In many forms, several genes that can cause the same phenotype or a closely related phenotype have been identified. In syndromic hearing loss, hearing loss is most frequently caused by dysfunction of the cochlea but the middle ear and the outer ear are also frequently involved. The most common form of syndromic hereditary SNHL is Pendred syndrome which is characterized by SNHL, bilateral dilatation of vestibular aqueduct with or without cochlear hypoplasia, and goiter. Majority of patients with Pendred syndrome have mutations in SLC26A4, and these mutations also cause nonsyndromic SNHL.[10,11] Pendred syndrome accounts for 3 % of all congenital hearing loss and mutations in SLC26A4 including those causing nonsyndromic SNHL account for 7 % of all deaf children at age of 4 years.[2] SLC26A4 encodes a chloride-iodide cotransporter and is critical for maintaining endolymphatic ion homeostasis, which is essential to normal inner ear function.

Mutations in mitochondrial DNA are rarely detected in congenital hearing loss, but its prevalence in patients with SNHL increases with aging. A1555G or A3243G mitochondrial DNA mutations are found in approximately 6 % of adult patients with SNHL without known causes, and both mutations cause cochlear dysfunc-

tion.[12,13] A3243G mitochondrial DNA mutation cause not only nonsyndromic SNHL but also syndromic SNHL such as MELAS (mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes) and MIDD (maternally inherited diabetes and deafness). A1555G mitochondrial DNA mutation causes rapidly progressive SNHL which leads to severe degree in patients with the onset of SNHL before age 10 and slowly progressive or nonprogressive SNHL which leads to mild to moderate degree in patients with the onset after age 10.[14] A3243G mitochondrial DNA mutation causes progressive SNHL which leads to moderate to severe degree in patients who developed hearing loss during adulthood.

Unique Clinical Aspects of Hereditary Hearing Loss

Hereditary hearing loss is unique compared to other hereditary diseases in the following three points. First, a large number of genes are involved in hereditary hearing loss, which makes it very difficult to identify causes and pathological mechanism in clinical practice. Second, without speech and language rehabilitation, hearing loss not only impedes audition but also hampers normal development of speech and language. Without speech and language, it is almost impossible to maintain good social relationship in the society of people with normal hearing. Thus, educational intervention is required in addition to medical intervention for children with SNHL. Third, congenital deaf children can learn and manage to communicate with others if early diagnosis of hearing loss followed by adequate rehabilitation can be made. Even severe hearing loss can be restored very effectively by hearing aids or cochlear implants coupled with early rehabilitative training in patients with hereditary hearing loss.[15] In most hereditary diseases, this level of functional restoration has not been possible yet. This feature lead to the worldwide implementation of universal newborn hearing screening which aims to screen neonates for hearing loss immediately after birth or before hospital discharge so that intervention can be initiated to prevent delayed language acquisition. Because of these unique clinical aspects of hereditary hearing loss, genetic testing of SNHL has high value in the otological approach to this disorder. Identification of genetic causes provides a key to understand the mechanism of hearing loss, leads to better management of hearing loss, and facilitates functional recovery by effective rehabilitation.

Strategy for Genetic Testing of Hearing Loss

Genetic testing of SNHL is conducted in several institutes worldwide including our institute, and the strategy is various among different institutes. In our institute, it consists of the following 3 steps; 1) identification of candidate patients who are suspected of having hereditary hearing loss, 2) identification of candidate genes to be tested, and 3) identification of causative mutations in the suspected genes.

Our criteria for candidate patients are patients presenting with bilateral hearing loss without known causes except for heredity. Unilateral hearing loss is included only when hearing loss is associated with specific types of anomaly in the inner ear, middle ear, or outer ear.

Candidate genes for syndromic hearing loss are determined by clinical diagnosis of syndromic hearing loss based on associated clinical symptoms. Usually, only one or a few candidate genes are responsible for each syndrome. Syndrome may be classified into subclasses based on the different expression of phenotypes, and diagnosis of subclasses may further narrow down candidate genes. On the other hand, it is very difficult to determine candidate genes for nonsyndromic hearing loss, and often impossible because of a large number of causative genes for a relatively undistinguishable phenotype, i.e. SNHL. Part of deafness genes for nonsyndromic hearing loss demonstrates unique auditory features or other clinical features in CT imaging of inner ear, electrophysiological testing, or inheritance pattern. For those genes, we are making an algorithm indicating the genes which should be tested and the order of the genetic tests based on clinical features and the results of genetic tests. After all the clinical examinations and tests for hearing loss, we determine the candidate genes and the order of genetic analysis according to the established algorithm (**Fig. 1**). This strategy is named systematic genetic testing for deafness, and tentative algorithm is currently used in our institutes to evaluate the sensitivity, specificity, and efficiency for clinical use.

Identification of causative mutations is mostly done with direct sequencing of the candidate genes using DNA extracted from blood samples. All exons and its flanking short sequences in introns are sequenced and analyzed for mutations. For large genes in which pathological mutations are mostly distributed within the restricted regions, sequencing may be done for the restricted region. In contrast, for several large genes with ubiquitous distribution of pathological mutations over entire region, screening by degenerate HPLC are first conducted, and sequencing analysis can be done only for the regions which showed abnormal screening results. For a few genes in which mutations are limited to only one or two frequent changes, restriction fragment length polymorphism PCR analysis is performed to detect the specific mutations. With the astonishing progress in the speed of sequencing machines, sequencing of whole human genome will be practically available in several years, first in laboratories, then in clinics. This may fundamentally change the way of genetic testing for SNHL.

Feedback to Patients

Discovery of many deafness genes had a significant

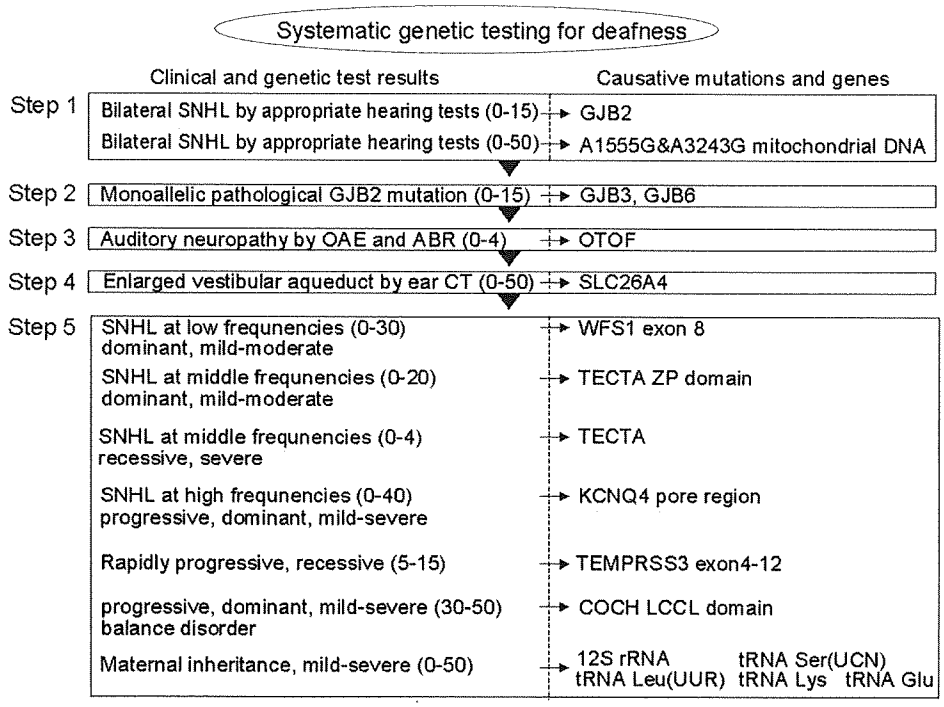

Fig. 1 Our original algorithm for systematic genetic testing for deafness in patients who are suspected of nonsyndromic SNHL (sensorineural hearing loss). Based on the clinical or genetic test results shown in the left column, candidate mutations or genes listed in the right column are determined. Corresponding mutations or genes for each clinical or genetic category are indicated by horizontal arrows. Genetic tests start from Step 1. If causative mutations are not determined or an indicated category does not fit for a patient, genetic tests proceed to the next step until causative mutations are determined or no appropriate category is found. Genes examined for specific exons, regions or domains are described with short explanatory tags, and those examined for all exons are described without explanation. Numbers in parenthesis indicate periods of age at onset of SNHL.

impact on the otological approach to patients with SNHL. First, explanation of the cause of SNHL to deaf patients or parents of deaf children has become possible in many cases. Without definite explanation, patients tend to visit other hospitals seeking for explanation and repeat redundant tests or treatments and feel anxiety about what is related to deafness of themselves or their children and whether other disability is also present but not detected. These lead to delay of rehabilitation which should be initiated immediately after diagnosis of hearing loss for effective acquisition of language and speech.[16] Thus, early and definite explanation by genetic tests facilitates rehabilitation.

Second, identification of causative mutations helps doctors to predict auditory features such as audiogram of the patients and prognosis of their hearing, especially in children who cannot cooperate with subjective hearing tests. This provides valuable information in making adequate planning of clinical follow-up, estimation of hearing levels for fitting hearing aids, and selection of occupation by patients.[17]

Third, prevention of deafness can be done by avoiding use of specific drugs or specific activities in genetically susceptible patients. As an example, patients with A1555G mitochondrial DNA mutation should avoid aminoglycosides which induce or aggravate SNHL by even one injection in subjects with this mutation.[18] Another example is that detection of SLC26A4 suggests dilatation of vestibular aqueduct even in neonates who are usually not tested for inner ear anomaly by CT or MRI. Patients with this mutation should have temporal bone CT and patients who are found to have dilatation of vestibular aqueduct should avoid activities in which physical shock on their head is likely to occur. This is because such a shock tends to cause aggravation of SNHL in these patients.

Fourth, identification of causative mutations in patients with syndromic hearing loss enables prevention or early detection of associated symptoms. These examples include diabetes mellitus in patients with A3243G mitochondrial DNA mutation and goiter in patients with SLC26A4 mutations. Early detection and management of these associated symptoms help to prevent disorders related to the associated symptoms such as diabetic retinopathy for diabetes mellitus, and facilitate early recovery from symptoms such as hypothyroidism. Because

occurrence of associated symptoms may delay more than 10 years after the onset of SNHL, many patients and even doctors who see those patients cannot notice the association of the symptoms with SNHL, and unnecessary or even harmful tests tend to be done for the diagnosis. Thus, it would be worthwhile to understand the associated symptoms and prepare the risk of manageable disorders at the time of diagnosis of SNHL. In addition, genetic tests may be valuable in substituting more stressful tests. For an example, renal biopsy and/or skin biopsy are currently necessary for diagnosis of Alport syndrome which is a hereditary nephritis associated with SNHL, and this procedure usually requires hospitalization and has a certain physical risk. Mutations in COL4A3, COL4A4, COL4A5, and MYH9 are known causes of Alport syndrome, but genetic tests of these genes are currently rarely available as a clinical test mainly because of an extremely high cost. Several laboratories in the world including my laboratory offer these tests as a research basis. With remarkable advances in genetics, increase of sensitivity and specificity and decrease of costs for genetic analysis are in progress. In the near future, diagnosis of Alport syndrome may be first done by clinical genetic tests, and renal and skin biopsy may be avoided in many patients.[19]

Fifth, identification of causative mutations clarifies the cell types and nature of damages which are responsible for SNHL, which is especially important for indication of cochlear implant surgery. Because spiral ganglion neurons which are necessary for successful cochlear implants are well preserved in most types of hereditary hearing loss, identification of mutations in the deafness genes usually indicates good indication for cochlear implant surgery. This is most helpful in babies who cannot corporate detailed audiological tests for evaluation of SNHL.

Identification of causative mutations is also important for clinical management of patients with auditory neuropathy. Auditory neuropathy is a distinct type of SNHL which features normal outer hair cell function and abnormal activities of auditory neurons, and a relatively frequent cause of congenital SNHL (~15 %). Development of speech and language cannot be expected by hearing aids in congenital auditory neuropathy because of poor speech recognition inherent in this disorder. Either inner hair cells or spiral ganglion neurons are affected, but current clinical tests cannot distinguish these two types. Because normal spiral ganglion neurons are necessary for success of cochlear implants, pathology underlying SNHL needs to be determined in order to evaluate the indication of cochlear implant surgery. Recent studies have shown that mutations in OTOF cause auditory neuropathy by inner hair cell dysfunction and that spiral ganglion neurons are normal in patients with these mutations.[20] In agreement with the pathological mechanism of mutations in OTOF, results of cochlear implants have been successful.[21] According to the recent studies, OTOF mutations may account for majority of congenital auditory neuropathy.[22] Thus, the genetic test for OTOF mutations in patients with auditory neuropathy is of high clinical importance.

Sixth, identification of causative mutations significantly helps to provide adequate genetic counseling which primarily concerns planning of pregnancy and delivery with the information of a recurrence risk. Prenatal genetic diagnosis of nonsyndromic SNHL is not conducted in most countries because of ethical issues. For syndromic SNHL which is associated with severe symptoms other than SNHL, prenatal diagnosis may be considered.

Future Expectation of the Use of Genetic Testing in Therapeutics

Although hearing aids or cochlear implants can significantly restore hearing in patients with SNHL, quality of restored hearing is quite different from original or normal hearing. These instruments are made to help remaining functions of the damaged inner ear, but future therapeutics aims at complete recovery of the inner ear. Because current clinical diagnostic modalities cannot identify which parts or cells in the inner ear is damaged, therapeutic approach targeting at specific parts or cells in the inner ear has not been used. Identification of damaged cells in the inner ear and the underlying mechanism by genetic testing undoubtedly facilitates development and introduction of novel and specific therapies to distinct types of SNHL.

As one of such therapies, we have established novel therapeutic approaches targeting at cochlear fibrocytes which are essential for normal hearing and involved in various type of SNHL including certain types of hereditary SNHL, age-related SNHL, noise-induced SNHL, and Meniere's disease. A rat model of SNHL due specific to cochlear fibrocytes was made by treatment with a mitochondrial toxin, 3-nitropropionic acid (3-NP), at a round window of inner ear.[23] Histological and molecular analysis in this model revealed caspase-mediated apoptosis in the cochlear fibrocytes.[24] As the therapy during acute phase of SNHL due to damages on cochlear fibrocytes, we used a general administration of caspase inhibitor, Z-VAD-FMK, to inhibit apoptosis.[25] This chemical, when administered before 3-NP treatment, almost completely inhibited 3-NP induced apoptosis of cochlear fibrocytes without obvious side effects and significantly improved the hearing level. Administration of Z-VAD-FMK after 3-NP treatment also showed significant inhibition of apoptosis and improvement of hearing. As the therapy during chronic phase of SNHL due to damages on cochlear fibrocytes, we used transplantation of bone marrow-derived mesenchymal stem cells into the inner ear in this animal model.[26] Histological examination of the transplanted rats demonstrated that transplanted stem

cells survived, migrated to the damaged area, and apparently substituted the damaged cochlear fibrocytes. Those stem cells made a connection with the surrounding fibrocytes and expressed connexins which are essential for reestablishment of potassium recycling pathway mediated by cochlear fibrocytes within the cochlea. Evaluation of hearing by auditory brainstem responses in the transplanted rats revealed significant improvement of hearing compared to control rats. These animal experiments indicate that therapeutic strategy for genetic SNHL may be personalized, based on the cause of SNHL, using chemicals targeting at specific molecules or stem cells targeting at specific tissues for regenerative therapy. In addition, novel therapies developed for genetic SNHL may be applicable to other types of SNHL with similar pathological features.

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Auditory neuropathy spectrum disorderの乳幼児例におけるASSR閾値

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要旨：Auditory neuropathyは2008年の国際会議からANSDと呼称されており，今回はその定義に従って診断されたANSDの乳幼児9例について検討した。後にABRが正常化していくようなみかけ上の難聴例（auditory immaturity）は除外した。経過をみていくうちにDPOAEが消失した5例はANSDとみなした。ASSRの閾値にはかなり大きなばらつきがあり，ANSDの病態が多彩であることが推定された。良聴耳のASSR閾値とCOR閾値を比較したところ，500～4000Hzでは有意な相関が認められた。ANSDの場合も補聴器装用効果をASSRでとらえることができ，推測された利得は平均でみてCORとの差は10dB以下であった。3例はASSRの3分法平均の閾値が70dBHL未満で，その場合CORの平均閾値も88dBHL以下と他症例より良好であったが，これらはすべて基礎疾患を伴っていた。ASSRおよびCOR閾値が100dBHL以上の重度難聴の例のうち2例に*OTOF*遺伝子変異が認められた。ASSRはANSDで行動聴力検査が不確実な場合に聴力および補聴器装用効果を評価する方法になりうるものと考えられた。

ーキーワードー

乳幼児聴力検査，聴性脳幹反応，歪成分耳音響放射

はじめに

Auditory neuropathyは耳音響放射（OAE）が正常で聴性脳幹反応（ABR）が無反応あるいは異常となる病態で，聴力に比し語音聴取力が低いことが特徴とされているが，その臨床像はさまざまであり，2008年に公表されたガイドラインではauditory neuropathy spectrum disorder（以下ANSDと略）と呼称されることになった[1)]。ANSDについては補聴器の効果あるいは人工内耳手術の適応などまだ意見の一致がみられていない点が多いため，今回はANSDの乳幼児例についてASSR検査を行い，他の所見と対比検討したので報告する。

対象と方法

平成18年4月～平成21年3月に国立成育医療センター耳鼻咽喉科を受診した新生児・乳幼児で，DPOAEの反応が両側正常かつABR閾値が両側80dBnHL以上でANSDとして扱った症例のうち，当院で療育・聴覚管理を行うことになった9例にASSR検査を行った。NICU児では中枢系の未成熟のためにABRの閾値上昇・波形分離不良がみられることがあり，ANSDと診断されてもABRが発達とともに正常化することがある。Bergらの報告[2)]ではNICU児の24%にANSDがみられているが，我々の検討[3)]ではNICU児でABR閾値上昇がみられた場合，19%は1歳時に20dB以上閾値が改善している。今回はそのような例は除外するために，1

歳を過ぎてもABR閾値が改善しないもの，あるいは初回のABR検査が1歳以上で行われたものを対象とした。またOAEが初期に正常でその後に消失した場合はANSDに含めるとされているので[1]，経過をみていくうちにDPOAEが消失した例も対象に含めた。ANSDの診断にはMRIにて蝸牛神経欠損を除外する必要があるものとされているが[1]，MRIまたはCTにて蝸牛神経欠損と考えられた例は除外した。なお，今回の検討例では9例中7例にMRIまたはCTを行っている。

ABRは日本光電MEB-2204（Neuropack）により測定した。鎮静下に検査を行い，刺激にはクリック音を用いて10dBステップで閾値を求めた。DPOAEはOAE analyzer ER-32（Grason-Stadler社製）またはILO292（Otodynamics社製）を用いて記録した。DPOAEの刺激音圧はL1＝65dB SPL，L2＝55dB SPLで，また測定条件はOAE analyzerおよびILO292ともにデフォルトの設定通りとした。両耳ともOAE analyzerでpassと判定されたもの，あるいはILO292で測定9周波数（1～6kHz）のうち8周波数以上がノイズレベルより5dB以上高いものをDPOAE正常とした。対象者の概略を表1に示すが，月齢は平均7.7ヶ月（±8.1 SD，SDは標準偏差），性別は男児5例，女児4例であった。基礎疾患として，9例のうち6例に難聴のリスクファクターが認められた。ABR閾値は7例がクリック105dBnHLで両側無反応で，1例が両側90dBnHL，1例が右80dBnHL，左100dBnHLであった。

ASSR検査にはGrason-Stadler社製Auderaを使用した。ASSRの刺激音は250，500，1k，2k，4kHzのAM/FM複合音を用い（変調周波数はそれぞれ67，74，81，88，95Hz），鎮静下に検査を行った。ASSR検査は年齢が7ヵ月～4歳のときに測定した。ABRおよびASSR検査ともに鎮静はトリクロホスナトリウム内服で行い，十分な鎮静が得られない場合は抱水クロラール坐薬を追加した。ASSRは推定聴力レベルではなく，実際の閾値（反応の得られた最小の刺激音圧）について検討したが，250Hzでは110dB HL，500Hzでは120dB HL，1～4kHzでは125dB HLで反応がなければ無反応とした。またASSRは10dBステップで閾値を求めたが，1～4kHzについては120dB HLで反応がない場合，125dB HLでも測定を行った。

さらに条件詮索反応聴力検査（COR）による聴覚評価も行い，比較検討した。COR検査の値は症例8を除いては，2～3歳での測定値を集計した。ASSRの方がCORよりやや行った時期が早い例が多いが，CORについては検査の精度を高めるため2～3歳での値をとっている。症例8は年齢が1歳6ヵ月より前のため，CORは1歳時に測定した値である。CORは250Hzでは95dB HL，500Hzでは100dB HL，1～4kHzでは110dB HLで反応がなければ無反応とした。

また，全例に補聴器装用を行っているが，7例には補聴器を装用してのASSR検査を行った。片耳ずつ補聴器を装用し，ASSRに外付けしたスピーカ（FE207E）から刺激音を提示し，ASSR測定を行った。なおスピーカは1m離れたところにおき，音圧校正を行ってから測定を行った（自由音場でのセットとして設定）。補聴器装用時のASSRについても閾値につき検討したが，250Hzでは70dB，500Hzでは80dB，1kHzでは85dB，2，4kHzでは90dBで反応がなければ無反応とした。補聴器両耳装用時のCOR閾値も全例で測定しているので，補聴器装用でのASSR検査を行った7例につき比較検討を行った。

結　果

DPOAEは症例9（初診が平成21年）を除き反復して測定を行っているが，5例は経過をみているうちにDPOAEの反応が消失した（表1）。DPOAEの反応が消失した5例のうち基礎疾患があるものは4例で，DPOAEが保たれている4例のうち基礎疾患があるものは2例であった。

図1に9例18耳についてのASSR閾値の分布を示す。大きなばらつきがあることがわかった。無反応の場合は閾値として最大の測定音圧に＋5dBした値をとり，3分法平均（500，1k，2k Hz）のASSR閾値を求めたが（表1，図1），うち3例6耳は3分法平均の閾値が70dBHL未満であった。これは症例1，5，7であり，すべて基礎疾患を伴っている例であった。うち2例はDPOAEが経過で消失していた。ASSR閾値（左右別）およびCOR閾値の平均を比較したものを図2に示す。CORについて

表1 症例の概略
DPの経過で＋はDPが保存されていることを示す。
ASSR閾値は3分法平均の値を示す。

症例	受診時月齢	性	基礎疾患	ABR	DPの経過	ASSR右閾値	ASSR左閾値
1	6	F	TTTSドナー，超低出生体重児	無反応	消失	63(dB)	57(dB)
2	6	M	なし	無反応	消失	100	107
3	5	M	超低出生体重児，高ビリルビン血症	無反応	消失	113	107
4	0	M	なし	無反応	＋	103	113
5	5	F	超低出生体重児，脊髄空洞症	両側90dB	消失	67	53
6	5	F	超低出生体重児，脳性麻痺	無反応	消失	128	127
7	10	M	West症候群，脳性麻痺	無反応	＋	67	67
8	5	M	高ビリルビン血症（核黄疸）	右80dB，左100dB	＋	110	110
9	28	F	なし	無反応	＋	97	107

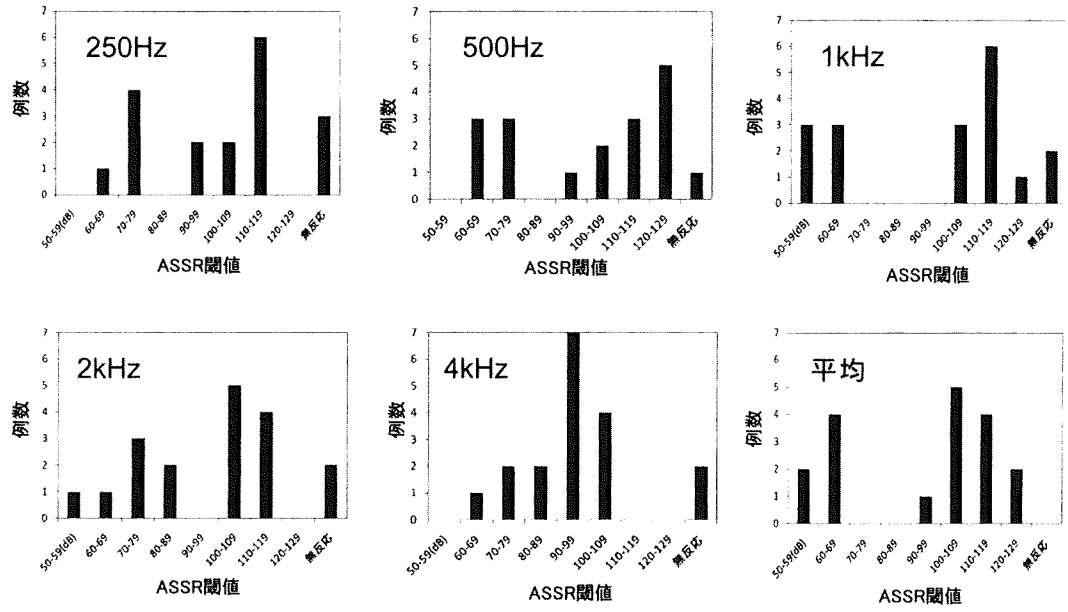

図1 周波数別にみた裸耳でのASSR閾値の分布（9例18耳）
ばらつきが大きく，3例6耳の閾値は平均が70dBHL未満である

も，無反応と判定した場合は最大の測定音圧に＋5dBした値を集計した。1～4kHzについてはCORの方が測定の最大音圧が小さいにもかかわらず，ASSRより閾値の平均値が大きかった。CORの3分法平均（500，1k，2k Hz）の閾値は9例の平均が96dBHLであるが，ASSR閾値が良好（3分法平均が70dBHL未満）の3例についてはCORの平均閾値も80，70，88dB HLと他症例より良好であった。ASSRとCORの閾値の相関をみるために，ASSRの左右別の閾値のうち良好な方の値とCOR閾値を周波数別に比較してみた。図3に全測定周波数の結果をまとめた散布図を示す。良聴耳のASSR閾値とCOR閾値の相関係数は，250Hzが0.616，500Hzが0.836，1kHzが0.922，2kHzが0.769，4kHzが0.755で，500Hz，1kHzについては有意水準1％，2kHz，4kHzについては有意水準5％で有意な相関が認められた。

ASSR閾値は7例14耳で補聴器装用時について自由音場で測定を行ったので，結果を図4に示す。裸耳のときと同様にばらつきはかなり大きかった。補聴器装用時のASSR閾値の3分法平均が50dBHL未満のものが4耳みられたが，これは症例1，5の結

果（２例４耳）であり，裸耳の ASSR 閾値が良好なものは補聴器装用時の ASSR 閾値も良好であった。なお症例７は補聴器装用時の３分法平均 ASSR 閾値が左右とも 60dBHL であった。また図５に補聴器装用での左右別 ASSR 閾値および COR 閾値の平均を比較したものを示すが，これでは差はほとんどなかった。ASSR および COR について裸耳の閾値から補聴器装用時の閾値を差し引くことにより推定した補聴器の利得の各周波数での平均を図６に示す。なお，補聴器装用時の閾値がスケールアウトの場合は利得を０として計算した。500Hz～4kHz については，平均で 15dB 以上の利得が ASSR および COR ともにみられた。COR の方が ASSR より推定される利得が良好な傾向があったが，その差は 10dB 以下であった。

考　察

ANSD は OAE が正常で ABR が無反応あるいは異常となる病態で，当初は auditory neuropathy ある

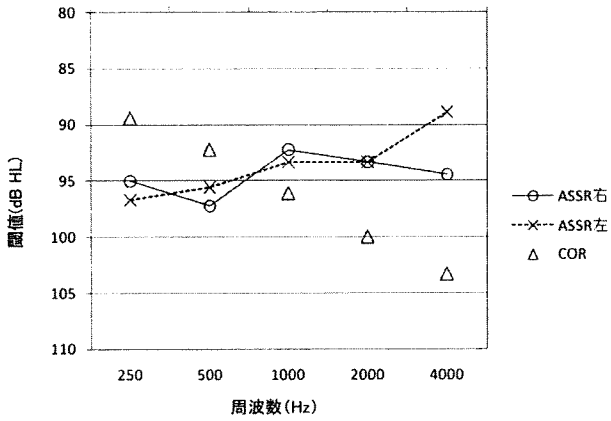

図２　裸耳での ASSR 閾値および COR 閾値の平均
COR の平均値は高音漸減型となった。

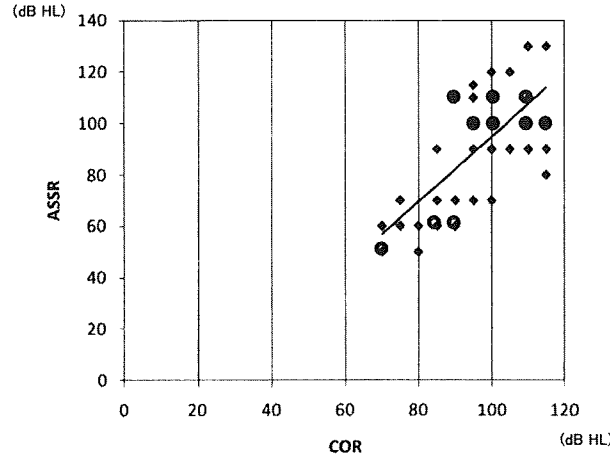

図３　ASSR 閾値と COR 閾値の比較
全データの分布を示す。図中に回帰直線を記した。
複数のデータが重なる点は●を重ねて示した。

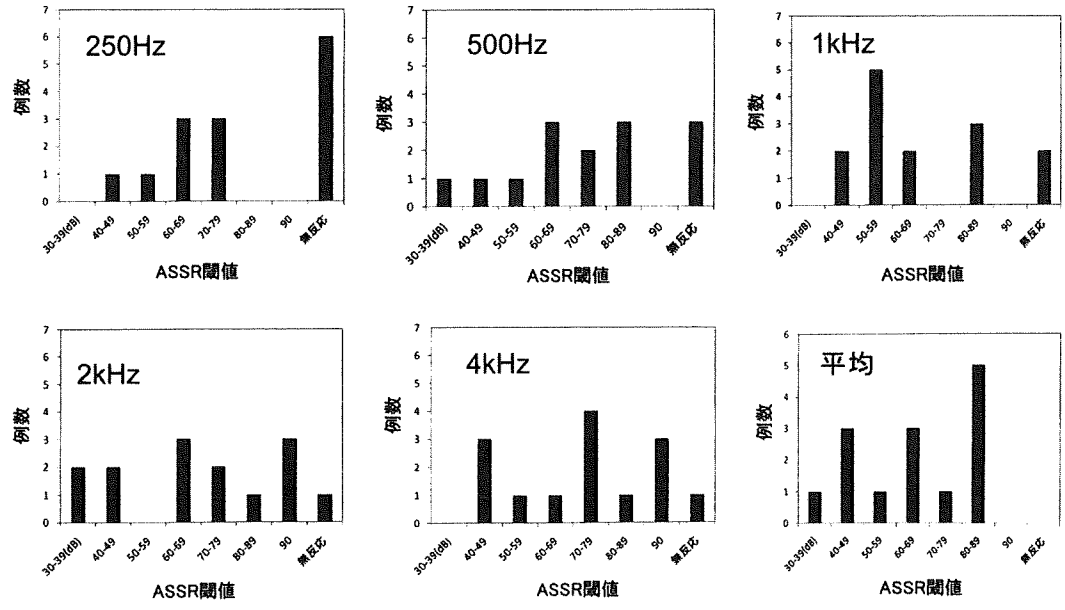

図４　周波数別にみた補聴耳での ASSR 閾値の分布（７例14耳）
裸耳と同様に症例ごとのばらつきが大きい。

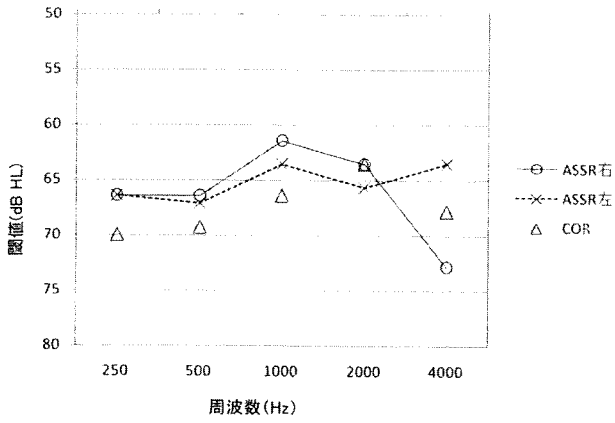

図5 補聴耳でのASSR閾値およびCOR閾値の平均
平均ではASSRとCORの閾値にあまり差はみられない。

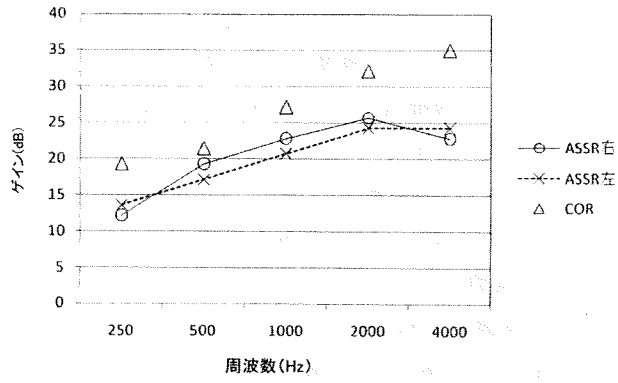

図6 ASSR閾値およびCOR閾値から推定された補聴器の利得の平均
推定される利得はCORの方がASSRよりやや大きい。

いはauditory nerve diseaseと呼ばれていたが[5,6]，2008年の国際新生児聴覚スクリーニング会議でANSDと呼称されることになった[1]。ANSDでは聴力障害の程度はさまざまであり，言語発達も正常のこともあれば全く語音が認識できず言語発達がみられないこともある[1]。聴力障害の程度に比べ語音聴取力が悪く，言語発達の良好な例でも雑音下では語音聴取が困難という特徴がある。なおABRが無反応であっても聴力がないということではなく[7]，ANSDには後にABRが正常化してくる例がある。そのようなABRでのみかけ上の難聴（髄鞘化不全などによる）はauditory immaturityとして真のANSDとは区別されるべきものとされており，今回はそのような例を除外するために対象は1歳時でABR無反応あるいは閾値が両側80dBnHLのものとした。

ANSDは外有毛細胞の機能が正常で聴覚の求心性神経経路の障害があるものと考えられている[1]。診断にはMRIにて蝸牛神経の欠損あるいは低形成を除外する必要がある[1,4]が，今回の9例のうち4例にはMRI（3-D CISS撮像）を行い蝸牛神経は正常であることを確認している。3例には側頭骨CTを行っており，いずれも内耳道・蝸牛神経管に異常はみられていないので蝸牛神経欠損は否定的である。残る2例（症例1，9）については画像検査を行っていないが，両側ともに蝸牛神経欠損である可能性は低いものと思われる。またOAEが初期に正常で後に消失した場合はANSDに含めるとされているので[1]，今回は経過をみていくうちにDPOAEが消失した5例もANSDとみなした。DPOAEが保存されているとした4例についても，症例8は年齢が1歳6ヵ月以下であり，2歳を過ぎて保たれているのは3例である。ANSDの概念は1996年より報告されているのに，新生児聴覚スクリーニングが開始（本邦ではモデル事業が2001年から）されてからANSDの報告が増えているのは，中途でOAEが消失する例が多いためかもしれない。

ANSDではABRやASSRで聴覚閾値を測定することは困難とされている[1]。今回はANSD例にAuderaを用いASSR測定を行ったが，図1に示すように閾値にはかなり大きなばらつきがあり，ASSRからもANSDの病態が多彩であることが推定された。80-Hz ASSRの起源もABRと同様に脳幹と考えられているが，その機序は異なるものと推定されており[8,9]，そのためABR無反応例でASSR閾値がさまざまとなったものと思われる。ASSRはABRと異なり活動電位の同期を必要としないので[10]，ANSDでASSRが検出されることは十分あり得る[8]。左右別のASSR閾値およびCOR閾値の平均を比較したところ（図2），CORはASSRに比べて低音域では閾値が低く，高音域では閾値が高い傾向があった。250Hz，500HzでASSRの閾値が比較的高いのは他の感音難聴例でも同じ[8]であり，ASSRは位相の同期性の有無を確率的に判定するので周期の長い低音域では検出しにくいためと考えられる。青柳は500Hz以下で80-Hz ASSRの閾値と聴力レベルとの相関が低くなる理由として聴覚フィルタを想定している[9]。高音域でCORとASSRが異なる理由

は不明であるが，これはASSR閾値が実際より低いのではなく，CORの精度が児の発達遅滞（9例のうち4例に重複障害）により低くなり閾値が高くなったためとも考えられる。なお，以前に我々がANSD以外のABR無反応例についてASSR閾値を調べた結果では[8]，1kHzが最も閾値が低かった。

ASSR閾値がかなり良好な症例（両耳とも3分法平均の閾値が70dBHL以下）が3例あり，そのような例ではCORの平均閾値も88dBHL以下（それ以外の例はすべて96dBHL以上）と良好であり，ASSRとCORの閾値には一致した傾向がみられた。良聴耳のASSR閾値とCOR閾値を比較したところ（図3），ばらつきはあるものの正の相関があり，周波数別に相関をみると500Hz～4kHz（250Hz以外）では有意な相関が認められた。ASSR閾値が真の聴覚閾値を示すかどうかは今後さらに検討が必要であるが，行動聴力検査と高い相関があったということはANSDの聴力の指標になり得るものと考えられた。

ANSDに対する補聴器の装用効果がみられる例は限られているとされているが[1]，今回の検討例はすべて両耳に補聴器装用を行っている。聴能訓練を行い，2例（症例3，5）は年齢相応の言語発達が認められたが，3例（症例2，4，9）は合併疾患がないのに発語は全くみられていない。ANSDで後にOAEが消失する場合は補聴効果が期待できるとした報告[1]があるが，症例3，5は経過観察中にDPOAEが消失した。補聴器装用時のASSR閾値（表3）にはかなりのばらつきがあるが，裸耳のASSR閾値が良好な例（症例1，5，7）は補聴器装用時の閾値も良好（平均が左右とも60dBHL以下）であった。補聴器装用時のASSR閾値とCOR閾値の比較（図5）では，裸耳のときと違い低音域ではASSRの方がやや良好な傾向がみられたが，これは250Hz，500Hzでは音場検査でのASSRの最大音圧が小さく（それぞれ70，80dBHL）スケールアウトの値も小さくなったためと考えられる。補聴器装用効果については，図6に示すように平均でみてASSRでも十分にとらえられており，補聴器の評価としてのASSRの有用性が示唆された。図6でCOR閾値からみた2，4kHzでの補聴器の利得がかなり大きくなっているのは，この周波数帯での裸耳のCOR平均閾値が高いためである。なお，ANSDでは補聴器装用により静かなところでの語音の聞き取りは改善するが，雑音下での聞き取りは困難とされており[11]，今回示された補聴効果よりも言語獲得のための補聴器の有効性は低くなることが予想される。ANSDでの人工内耳の効果は他の重度感音難聴と変わらないので[7]，補聴器の効果が十分でないANSDでは人工内耳が検討されるべきであるが，前述したauditory immaturity（一過性のANSD）の可能性を考え手術適応の決定には行動聴力検査を含めた十分な聴力評価が必要である。

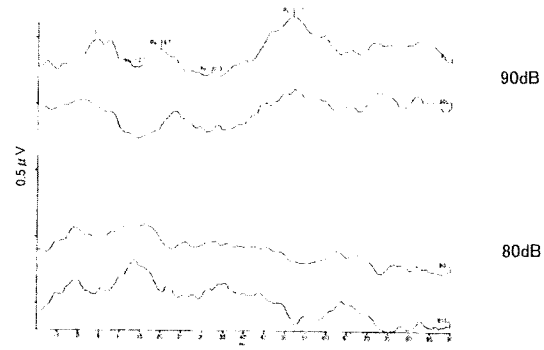

図7　ANSD例におけるCAEP検査の測定例
1000Hz，左耳での測定例で，90dBで明瞭に反応（MLR）が認められる。

なおANSDにおいてABR検査を行うときには極性を変えたクリック音（rarefaction, condensation）を用いることが推奨されているが[1]，今回はalternating clickで検査を行った。また，ANSDにおいて行動聴力検査の結果が不確かなときは皮質誘発反応（Cortical auditory evoked potentials：CAEPs）が有用であるとされており[1]，我々も3例にAuderaを用いてCAEPsの測定を行った。結果の例を図7に示すが，測定した3例はすべて発語のみられない例であったのにもかかわらずCAEPsは反応があり，本検査の意義は今後の課題である。ANSDでは*OTOF*，*PMP22*，*MPZ*，*NDRGI*などの遺伝子変異が報告されているが[11]，今回の症例では2例に*OTOF*遺伝子変異が認められた。*OTOF*遺伝子変異のみられた2例はASSRおよびCORの平均閾値がいずれも100dBHL以上の重度難聴であり，補聴器装用効果も少ないため1例は人工内耳手術を行った。

ま と め

1．Auditory neuropathyは2008年の国際会議からANSDと呼称されており，今回はその定義に従って診断されたANSDの9例について検討した。経過をみていくうちにDPOAEが消失した5例もANSDとみなした。
2．ASSRの閾値にはかなり大きなばらつきがあり，ANSDの病態が多彩であることが推定された。良聴耳のASSR閾値とCOR閾値を比較したところ，500Hz～4kHzでは有意な相関が認められた。
3．ANSDの場合も補聴器装用効果をASSRでとらえることができ，推測された利得は平均でみてCORとの差が10dB以下であった。
4．3例はASSRの3分法平均の閾値が70dBHL未満で，その場合CORの平均閾値も88dBHL以下と他症例より良好であった。これらはすべて基礎疾患を伴っている例であった。ASSRおよびCOR閾値が100dBHL以上の重度難聴の例のうち2例に*OTOF*遺伝子変異が認められた。

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Auditory steady-state response thresholds in infants and young children with auditory neuropathy spectrum disorder

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Auditory neuropathy, renamed by consensus at a recent international conference as auditory neuropathy spectrum disorder (ANSD), is a specific form of hearing loss defined by normal otoacoustic emissions, but severely abnormal or completely absent auditory brainstem responses. We investigated the distribution of auditory steady-state response (ASSR) thresholds in 9 infants and young children with ANSD. The large variability of ASSR thresholds indicated the heterogeneous nature of this disorder. Correlation values showed a significant positive relationship ($p<0.05$) between ASSR and conditioned orientation response audiometry (COR) thresholds at 500-4000Hz. To estimate the functional gains obtained from the use of hearing aids, we examined the dB difference between unaided and aided thresholds of ASSR and COR. The average functional gains estimated by the ASSR thresholds were up to 15 dB at 500-4000Hz, which were slightly lower than those estimated by the COR thresholds. ASSR testing is considered to be useful for hearing aid validation when behavioral test methods are inconclusive. ASSR may be useful for the estimation of residual auditory capacities and hearing aid benefits in infants and very young children with ANSD.

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