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

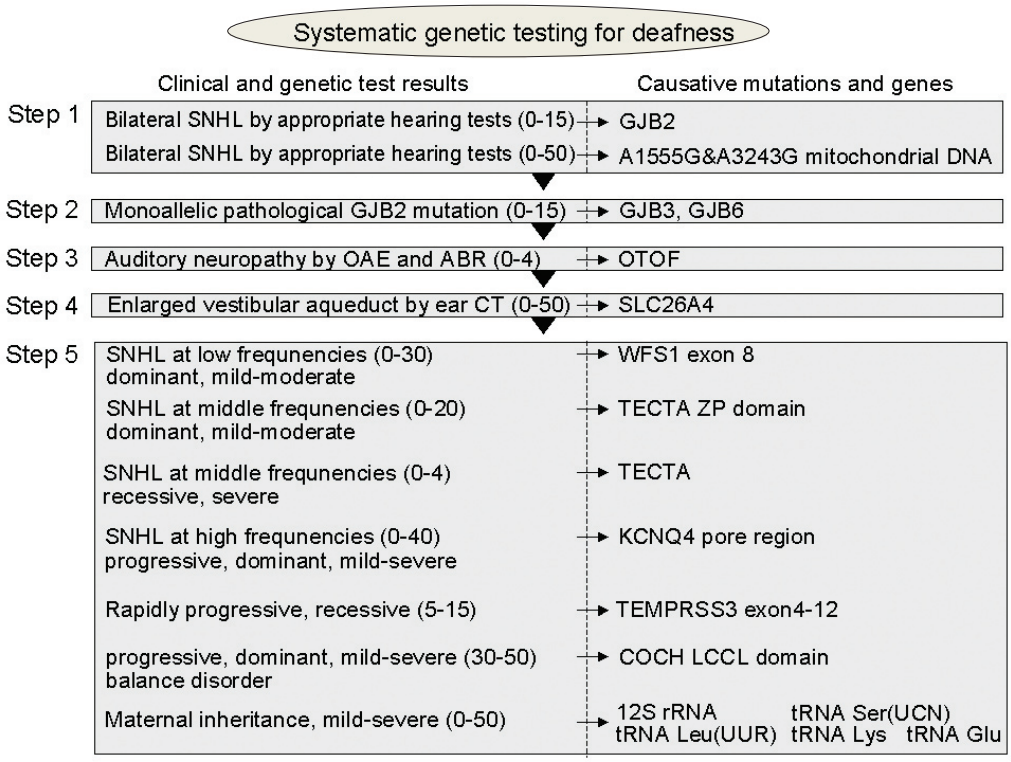

Fig. 1 Our original algorithm for systematic genetic testing for deafness in patients who are suspected of nonsyndromic SNHL (sensorineural hearing loss). Based on the clinical or genetic test results shown in the left column, candidate mutations or genes listed in the right column are determined. Corresponding 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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