Comprehensive Genetic Testing for Sensorineural Hearing Loss

A Guide for Clinicians

Cincinnati Children's

Hearing loss affects about 1 in 500 newborns, and a genetic etiology is suspected in two thirds of these patients.

Approximately 70% of children with a genetic basis for their hearing loss have isolated (nonsyndromic) deafness and the remaining 30% have hearing loss accompanied by other physical manifestations. Mutations in upwards of 100 genes are known to result in hearing loss which can be inherited in an autosomal dominant, autosomal recessive, X-linked, or mitochondrial (maternal inheritance) manner.

Most prelingual nonsyndromic hearing loss is inherited as an autosomal recessive disorder, accounting for about 80% of all genetic causes of hearing loss. To date, 42 different genes have been implicated in autosomal recessive nonsyndromic hearing loss [Duman et al 2012]. Mutations in some of these genes, such as *GJB2, MYO7A, CDH23, OTOF, SLC26A4, TMC1,* are quite common and can easily be tested for in individuals with hearing loss. Mutations in many other genes are extraordinarily rare, some of which have been reported in only one or two consanguineous families.

Elucidation of the genetic basis of hearing loss is crucial for management of patients and their families. Therefore, genetic testing is recommended by the American College of Medical Genetics and Genomics as an integral part of the evaluation of children with permanent hearing loss. The complex heterogeneity and indistinguishable phenotype caused by most of these genes is amenable to testing via multi-gene panels which detect mutations in many of the more common genes causing autosomal recessive nonsyndromic hearing loss. In children with hearing loss in combination with other features suggestive of a specific genetic syndrome, direct analysis of the gene(s) likely responsible for that condition is the most-cost-effective approach to genetic testing.

Benefits of Genetic Testing

Genetic test results may provide:

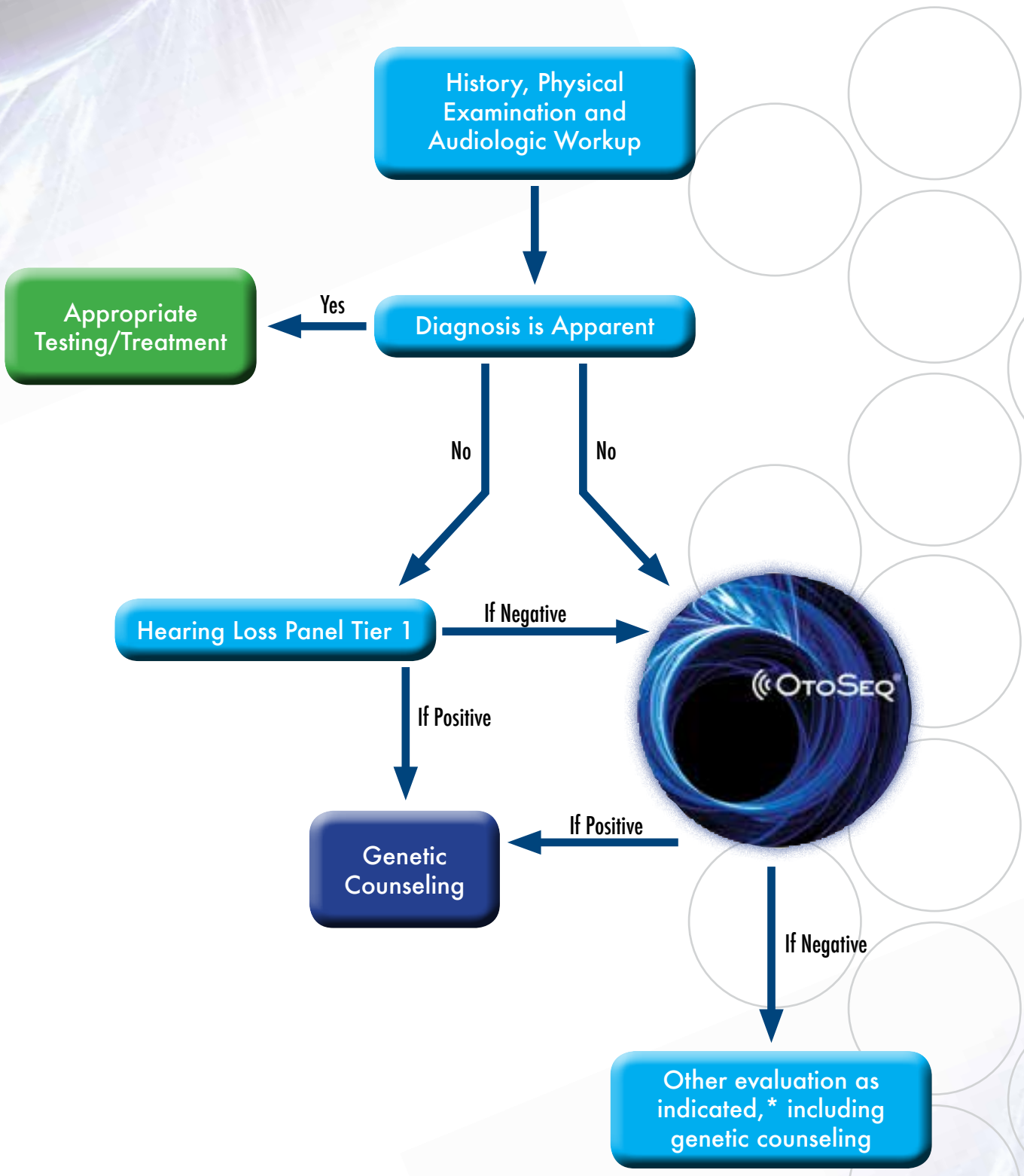
- Accurate determination of the etiology of the patient's hearing loss.
- Reduction or elimination of the need for further invasive and costly diagnostic tests.
- Basis for clinical prognosis including future hearing and potential medical complications.
- Guidance regarding treatment and long-term medical management, particularly in the young infant.
- Definitive information to guide genetic counseling of families.

Testing Options at CCHMC

We strive to provide clinicians with a broad range of testing options to fit the individual needs of their patients. Therefore, we offer:

- Hearing Loss Panel Tier 1—testing for mutations in *GJB2, GJB6*, *MTRNR1* and *MTTS1* which account for 40% of the genetic causes of hearing loss. Reflex testing to our OtoSeq® Hearing Loss Panel is an option for patients with normal Tier 1 results.
- OtoSeq® Hearing Loss Panel—our comprehensive next-generation sequencing panel which identifies an estimated 80% of the genetic causes of hearing loss.
- Disease specific panels for Usher syndrome, branchiootorenal spectrum disorder and Pendred syndrome.
- Individual gene sequencing or mutation detection for common causes of genetic hearing loss including: *CDH23, EYA1, GJB2, MYO7A, MTRNR1, MTTS1, OTOF* and *SLC26A4*. Testing for a common 324kb deletion involving *GJB6* is also offered.

Diagnostic Algorithm of a Child with Sensorineural Hearing Loss



** Indicated by history and physical exam. This may include an electrocardiogram, syphilis tests, autoimmune panel (i.e. ESR, Western blot), fasting glucose, urinalysis, thyroid function studies, electronystagmography, ophthamalogical exam and electroretinography.*

Hearing Loss Panel Tier 1

Mutations in *GJB2* (connexin 26) are the most frequent cause of autosomal recessive nonsyndromic hearing loss. Mutations in *GJB2* are found in various populations, with carrier rates of approximately 1 in 30 in the United States Caucasian population, and 1 in 20 in the Ashkenazi Jewish population. Large deletions involving *GJB6* are identified in 1% of North American patients with hearing loss, typically in association with a single *GJB2* mutation (digenic inheritance). Nonsyndromic hearing loss secondary to mutations in the *MTTS1* and *MTRNR1* genes accounts for about 1% of childhood hearing loss in the United States. Mutations in *MTRNR1* may be associated with aminoglycoside ototoxicity in some patients.

OtoSeq® Hearing Loss Panel,

our next-generation sequencing panel of 23 genes (see Table 1) associated with sensorineural hearing loss in childhood, is indicated for patients with hearing loss of unknown etiology. **OtoSeq®** testing is also a cost effective option for patients in whom one or more diagnoses are being considered. The **OtoSeq®** Hearing Loss Panel may also be used as follow-up testing in patients with normal *GJB2* or Hearing Loss Panel Tier 1 test results.

OtoSeq® was specifically designed to identify mutation(s) in the most common genes causing early onset sensorineural hearing loss, particularly those associated with other risk factors, while limiting the number of findings of uncertain clinical significance. Preliminary data suggest that **OtoSeq®** detects approximately 80% of the genetic causes of early onset sensorineural hearing loss.

Disease-Specific panels

Usher syndrome Panel (*CDH23, CLRN1, GPR98, MYO7A, PCDH15, USH1C, USH1G, USH2A, WHRN)*
Usher syndrome is characterized by sensorineural hearing loss in association with retinitis pigmentosa. Approximately 2% of patients with early onset sensorineural hearing loss have Usher syndrome, and at least half of all patients who are deaf and blind have Usher syndrome. The prevalence of Usher syndrome in the general population is approximately 4 per 100,000 and the carrier frequency is estimated at 1-in-70. Usher syndrome is often divided into subtypes based severity of symptoms and age at onset of retinitis pigmentosa. Usher syndrome is inherited as an autosomal recessive disorder. Genetic testing improves classification of individual patients which allows for improved prognostic information for patients and their physicians.

Usher syndrome type 1 (USH1) is characterized by severe to profound congenital sensorineural hearing loss, balance disturbances with poor coordination and retinitis pigmentosa with onset in childhood. **Usher syndrome type 2 (USH2)** is associated with congenital, bilateral sensorineural hearing loss which is quite variable and tends to affect higher frequencies, in addition to retinitis pigmentosa with onset in adolescence to adulthood. **Usher syndrome type 3 (USH3)** is associated with bilateral sensorineural hearing loss which is quite variable, is often progressive, and tends to affect higher frequencies, in addition to retinitis pigmentosa with onset in adolescence to adulthood.

Mutations in nine different genes are known to cause Usher syndrome, and testing for these can be performed

Table 1. OtoSeq® Hearing Loss Panel includes sequencing of all of the genes listed below.

Gene	Disorder
CDH23	DFNB12, USH1D
CLRN1	USH3A
EYA1	BOR/BOS1
FOXI1	Pendred syndrome
GJB2	DFNB1A, DFNA3A
GJB6	DFNB1B, DFNA3B
GPR98	USH2C
KCNJ10	DFNB4 and SeSame syndrome
MYO6	DFNA22, DFNB37
MYO7A	DFNB2, DFNA11, USH1B
OTOF	DFNB9, AUNB1
PCDH15	DFNB23, USH1F

Gene	Disorder
POU3F4	DFNX2 (DFN3)
SIX1	BOS3
SIX5	BOR2
SLC26A4	Pendred syndrome/DFNB4
TMC1	DFNB7/11, DFNA36
TMIE	DFNB6
TMPRSS3	DFNB8/10
USH1C	USH1C, DFNB18
USH1G	USH1G
USH2A	USH2A
WHRN	DFNB31, USH2D

on a clinical basis. Additional loci have been postulated which are not yet amenable to clinical testing. Genetic testing will identify mutation(s) in most patients with Usher syndrome (See Table 2). Gross deletions, duplications and complex genetic events, which are not amenable to a sequencing-based detection, are quite common in many of these genes [Zwaenepoel et al 2001]. Thus, additional testing may be warranted in patients for whom a single pathogenic mutation is identified (See Figure 2).

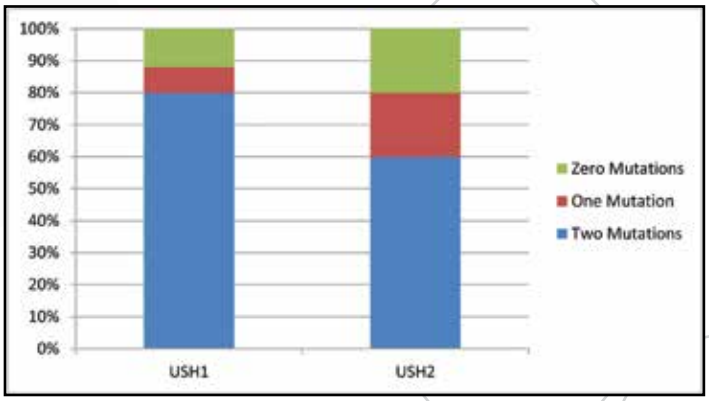
Mutations in *CDH23, MYO7A, PCDH15, USH1C* and *WHRN* are also associated with nonsyndromic hearing loss (see Table 1), typically inherited as autosomal recessive disorders. Both autosomal recessive and autosomal dominant inheritance has been demonstrated for nonsyndromic hearing loss secondary to mutations in *MYO7A*. Genotype/phenotype correlations are not certain; however, it has been suggested that missense mutations may confer a less severe phenotype and are more likely to segregate with nonsyndromic hearing loss while splicing and truncating mutations are more likely associated with USH1. Digenic inheritance has not been proven in USH1.

Table 2. Genes associated with Usher Syndrome Types 1, 2, and 3.

USH1	
Gene	**Diagnostic Yield**
CDH23	10%
MYO7A	50%
PCDH15	8%
USH1C	15%
USH1G	<1%
USH2	
Gene	**Diagnostic Yield**
USH2A	80%
GPR98	7%
WHRN	<1%
USH3	
Gene	**Diagnostic Yield**
CLRN1	50%

Diagnostic yield is estimated for each gene [Stabej et al 2012]. Diagnostic yield refers to the likelihood of finding a mutation in a specific gene in a patient with the associated syndrome.

Figure 2. Frequency of finding two mutations, one mutation, and zero mutations in genes associated with Usher Syndrome Type 1 or Usher Syndrome Type 2.



Branchiootorenal Spectrum Disorder Panel
(*EYA1, SIX1, SIX5*)
Branchiootorenal spectrum disorder is characterized by malformations of the external, middle and/or inner ear, branchial clefts or cysts, hearing loss which may be of sensorineural, conductive or mixed types, and in some patients, renal malformations of varying severity. Over 90% of patients with branchiootorenal spectrum disorder have hearing loss of some degree. Onset of hearing loss ranges from childhood to young adulthood. Progressive hearing loss occurs in about 30% of patients and may be associated with enlarged vestibular aqueducts. Branchiootorenal spectrum disorder has an estimated prevalence of 1/40,000, and is inherited as an autosomal dominant condition. This condition is highly variable, even among family members.

Approximately 40% of individuals with branchiootorenal spectrum disorder have an identified mutation in *EYA1*, 80% of which can be identified by this test. Mutations in *SIX5* and *SIX1* each account for 2-3% of symptomatic individuals. Additional, as of yet, unidentified genes may be responsible for branchiootorenal spectrum disorder in some families.

Pendred Syndrome Panel
(*SLC26A4, FOXI1* and *KCNJ10*)
Approximately one-third of all children with sensorineural hearing loss have an abnormality in their temporal bones. Mutations in the *SLC26A4* gene, which encodes for the pendrin protein, are identified in 25%-50% of children with temporal bone abnormalities and/or enlarged vestibular aqueducts.

Biallelic mutations in *SLC26A4* result in one of two phenotypes: Pendred syndrome (hearing loss in association with cochlear abnormalities, enlarged vestibular aqueducts and euthyroid goiter) or DFNB4 (nonsyndromic sensorineural hearing loss, vestibular dysfunction, and enlarged vestibular aqueduct without thyroid defects).

Biallelic mutations are identified in 80-90% of patients with a family history of PDS, while only a single mutation is identified in approximately 30% of patients with no family history of PDS.

Heterozygous mutations in two additional genes, *FOXI1* and *KCNJ10*, have been reported in association with DFNB4 in a few individuals in which only a single *SLC26A4* mutation was identified, thus supporting digenic inheritance as a rare cause of this disorder. Of note, biallelic inheritance of two mutations in *KCNJ10* causes SeSAME/EAST syndrome, which is characterized by seizures, sensorineural deafness, ataxia, mental retardation, and electrolyte imbalance.

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