Comprehensive Genetic Testing for Sensorineural Hearing Loss

A Guide for Clinicians
**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, MYO7A, CDH23, OTOF, SLC26A4, TMC1*, 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

History, Physical Examination and Audiologic Workup

- *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, ophthalmological exam and electroretinography.

Diagnosis is Apparent

- Yes: Appropriate Testing/Treatment
- No: Hearing Loss Panel Tier 1

Hearing Loss Panel Tier 1

- If Negative: Other evaluation as indicated,* including genetic counseling
- If Positive: Genetic Counseling

Genetic Counseling

- If Positive: If Positive
- If Negative: Other evaluation as indicated,* including genetic counseling

*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, ophthalmological 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.

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

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

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.
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.

<table>
<thead>
<tr>
<th>USH1</th>
<th>Diagnostic Yield</th>
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<tbody>
<tr>
<td>CDH23</td>
<td>10%</td>
</tr>
<tr>
<td>MYO7A</td>
<td>50%</td>
</tr>
<tr>
<td>PCDH15</td>
<td>8%</td>
</tr>
<tr>
<td>USH1C</td>
<td>15%</td>
</tr>
<tr>
<td>USH1G</td>
<td>&lt;1%</td>
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<table>
<thead>
<tr>
<th>USH2</th>
<th>Diagnostic Yield</th>
</tr>
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<tbody>
<tr>
<td>USH2A</td>
<td>80%</td>
</tr>
<tr>
<td>GPR98</td>
<td>7%</td>
</tr>
<tr>
<td>WHRN</td>
<td>&lt;1%</td>
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<table>
<thead>
<tr>
<th>USH3</th>
<th>Diagnostic Yield</th>
</tr>
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<tr>
<td>CLRN1</td>
<td>50%</td>
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</table>

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.
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.

**References**

**Nonsyndromic hearing loss**


Branchiootorenal (BOR/BOS) spectrum disorder


Usber syndrome


Pendred syndrome


