

Molecular Testing for Connexin 26 (*GJB2*) and Connexin 30 (*GJB6*) Mutations Associated with Non Syndromic Hearing Loss

The UNC Hospitals Molecular Genetics Laboratory offers DNA sequencing of the coding region of the connexin 26 (*GJB2*) gene and analysis of deletions in the connexin 30 (*GJB6*) gene to detect mutations that are associated with hereditary nonsyndromic hearing loss.

Biology of the disease:

Profound congenital hearing loss affects approximately 1 in 1000 infants. Approximately 50% of cases have a genetic cause, and 70% of these genetic cases are classified as nonsyndromic, meaning that hearing loss is the only clinical finding. The remaining 30% have additional signs or symptoms with over 400 different syndromes described. Familial nonsyndromic hearing loss (NSHL) is primarily sensorineural, with the mode of inheritance being 77% autosomal recessive, 22% autosomal dominant, and the remainder X-linked or mitochondrial. Several genetic loci have been mapped, including the *DFNB1* locus on chromosome 13q12 containing the gap junction beta 2 (*GJB2*) gene encoding connexin 26 protein and the *GJB6* gene encoding connexin 30 protein. Connexins 26 and 30, transmembrane proteins expressed in the inner ear, form ion channels regulating intercellular communication. Numerous disease-causing mutations in *GJB2* have been identified, the most common of which is 35delG, which is found in over two-thirds of Caucasians with *DFNB1*-linked hearing loss. It is estimated that 35delG is responsible for 10% of all hearing loss and 20% of all hereditary hearing impairment. Other mutations in *GJB2* are more common in other ethnic groups; for example, 167delT accounts for majority of cases of autosomal recessive NSHL in people of Ashkenazi Jewish ethnicity. NSHL can also result from compound heterozygosity of a *GJB2* mutation and a *GJB6* (connexin 30) deletion. Two deletion mutations have been identified in *GJB6* that are correlated with NSHL, one of 232 Kb and the other of 309 Kb. Each has been implicated in hearing loss when present on both alleles or in trans to a mutation in *GJB2*.

Clinical Indications for Connexin 26 gene mutation testing:

Molecular testing for connexin 26 and 30 gene mutations should be considered in individuals who have congenital or early-onset hearing loss but no other clinical findings, and in hearing-impaired individuals with a family history of hearing impairment.

Laboratory Testing for Connexin 26 and 30 gene mutations:

The preferred sample is EDTA- or ACD-anticoagulated blood (lavender-top or pale yellow top, 3mL), which may be refrigerated up to 48 hours before analysis. Molecular testing of *GJB2* is done by sequencing the coding region (exon 2) and flanking intronic regions. Results are reported as normal or mutation(s) detected. Any variant identified is interpreted as a deleterious mutation, a variant of unknown significance, or a polymorphism. Testing of *GJB6* is done by a multiplex PCR followed by fragment analysis. Three sets of primers, one each specific for the normal allele, the 232 Kb deletion and the 309 Kb deletion are used simultaneously in a multiplex reaction and the resultant PCR products are analyzed on a capillary electrophoresis instrument. Presence of amplicons of the corresponding size identify whether either of the two deletion mutations are present in the patient's sample. Results are reported as wild type or deletion detected. Detection of mutations has diagnostic and reproductive implications, while a negative test result does not exclude a genetic cause for

hearing loss.

Genetic counseling is recommended, and you may call (919) 966-2229 to make a patient appointment.

References:

1. <https://www.ncbi.nlm.nih.gov/books/NBK1536/>
2. ACMG Statement: Genetic evaluation guidelines for the etiologic diagnosis of congenital hearing loss. *Genetics in Medicine*, 2002; 4: 162-171 (website: <https://pubmed.ncbi.nlm.nih.gov/12180152/>)
3. del Castillo et al. A novel deletion involving the connexin-30 gene, del(*GJB6*-d13s11854), found in *trans* with mutations in the *GJB2* gene (connexin-26) in subjects with DFNB1 non-syndromic hearing impairment. *Journal of Medical Genetics* 2005;42:588-594.

Questions?

Call the UNC Molecular Genetics Lab at ((984) 974-1825 or

Dr. Jessica Booker at (984) 974- 1456

E-mail: Jessica.Booker@unchealth.unc.edu