Chapter 11

Deafness

Richard J.H. Smith and Guy Van Camp

Introduction

Recent advances in the molecular biology of hearing and deafness are being transferred from the research laboratory to the clinical arena. This transfer of knowledge is enhancing patient care by facilitating the diagnosis of hereditary deafness. Traditionally, hereditary deafness has been distinguished from nongenetic causes of deafness by otologic, audiologic, and physical examinations, complemented by a family history and ancillary tests such as temporal bone computed tomography, urinalysis, thyroid function studies, ophthalmoscopy, and electrocardiography. Even using this test battery, an unequivocal distinction between genetic and nongenetic causes of deafness often is difficult. If comorbid conditions are identified, the deafness may fall into one of more than 400 recognized types of syndromic hearing loss, but if hearing loss segregates as the only abnormality, diagnosing the deafness as nonsyndromic and inherited is challenging.[1]

The relative contributions of syndromic and nonsyndromic deafness to the total deafness genetic load vary with age of ascertainment. Because syndromes are generally straightforward to recognize, most are noted at birth. In aggregate, syndromes account for about 30% of prelingual deafness. The majority of congenital hereditary deafness, however, is nonsyndromic, and this relative contribution increases with age, reflecting the greater occurrence and diagnosis of postlingual nonsyndromic as compared to postlingual syndromic deafness in late childhood and adulthood.

In many cases, the diagnosis of autosomal recessive nonsyndromic deafness (ARNSD) is a diagnosis of exclusion. Current data suggest that even with a thorough history and physical examination, in simplex families (only one affected child) errors in diagnosis are made one third of the time; that is, a child is diagnosed with ARNSD when the correct diagnosis is congenital acquired deafness.[2] This type of error precludes appropriate genetic counseling and can be a source of concern and anxiety for the family.

Molecular genetic testing offers a potential means to unequivocally diagnose inherited deafness. Allele variants of many genes are known to cause hereditary deafness;[3] however, extreme heterogeneity and the relatively small contribution each gene makes to the total deafness genetic load make it impractical to offer complete mutation screening of all genes known to cause inherited deafness. Currently, clinical mutation screening is offered for three genes that cause nonsyndromic hearing loss and deafness (DFNB), *GJB2*, *SLC26A4*, and *WFS1*, and one gene that causes syndromic deafness, *EYA1*. The clinical utility of screening *GJB2* and *SLC26A4* is based on three facts. First, both genes cause types of deafness that are difficult to diagnose without genetic testing (*GJB2* for DFNB1; *SLC26A4* for DFNB4 and Pendred syndrome); second, the relative contribution of these two genes to the total genetic deafness mutation load is high; and third, both genes are relatively easy to screen by molecular methods. Mutation screening of *WFS1* (nonsyndromic autosomal dominant deafness, DFNA6/14) is offered because the audioprofile of affected persons is unique, and in the case of *EYA1*, the occasional association of branchio-oto-renal syndrome with prenatal lethality has been the impetus to develop molecular genetic testing. In this chapter, genetic testing of these four genes is reviewed.

DFNB1(*GJB2*)

Molecular Basis of Disease

In 1994, Guilford et al. mapped the first locus for ARNSD to chromosome 13q12-13 and named it DFNB1 (Online Mendelian Inheritance in Man [OMIM; database online] #220290).[4] Three years later, the deafness-causing gene at this locus was identified as *GJB2*.[5] *GJB2* encodes a transmembrane protein called Connexin 26 (Cx26) that oligomerizes with five other connexins to form a connexon. Connexons in adjoining cells join to form gap junctions, or conduits, that facilitate the rapid exchange of

electrolytes, second messengers, and metabolites from one cytoplasm to another.[6] Interestingly and unexpectedly, mutations in Cx26 have been found in approximately 50% of persons with severe-to-profound congenital ARNSD in several worldwide populations.[7–11]

Immunohistochemical studies of Cx26 expression in rat cochleae have demonstrated that two groups of cells are interconnected via gap junctions. The first group, nonsensory epithelial cells, includes interdental cells of the spiral limbus, inner and outer sulcus cells, sensory supporting cells, and cells within the root process of the spiral ligament. The second group, the connective tissue cell gap junction system, includes fibrocytes within the spiral ligament and spiral limbus, basal and intermediate cells of the stria vascularis, and mesenchymal cells, which line the scala vestibule and interconnect the two populations of cell types. Expression of Cx26 in the vestibular labyrinth is similar.[12,13] These studies suggest that the Cx26 gap junction system plays a role in potassium recycling, facilitating the rapid transport of K^+ ions through the supporting cell network to the stria vascularis, thereby helping to maintain the unique potassium-sodium endolymph balance.[14]

Although more than 80 different deafness-causing allele variants of *GJB2* have been reported,[15] in populations of European descent a single mutation predominates, 35delG. This mutation reflects the deletion of one deoxyguanosine from a string of 6, resulting in a shift in reading frame and premature protein truncation. Based on an analysis of single nucleotide polymorphisms (SNPs) tightly linked to the 35delG mutation, this mutation segregates on a common haplotype background and arose as a result of a founder effect about 10,000 years ago.[16]

Today, the carrier frequency for the 35delG mutation in the midwestern United States is approximately 2.5%, and in this population roughly two thirds of persons with Cx26-related deafness are 35delG homozygotes.[2,17] Of the remaining persons with Cx26-related deafness, most are 35delG heterozygotes, and carry a second, noncomplementary mutation. Consistent with a founder origination is the observation that in some populations the 35delG mutation is rare. For example, in the Ashkenazi Jewish and Japanese populations, the 167delT and 235delC mutations, respectively, are most common.[18,19]

Clinical Utility of Testing

Establishing a molecular diagnosis of *GJB2*-related deafness is important clinically since children with this type of deafness can avoid further diagnostic tests and are not at increased risk for medical comorbidity. Bony abnormalities of the cochlea are not part of the deafness phenotype, and developmental motor milestones and vestibular function are normal.[11,20,21] The rare exceptions include a child with bony cochlear overgrowth noted at surgery,[22] a child with asymmetry of the right modiolus,[22] a child with vertigo, migraine, and unilateral weakness,[11] and a child with marked prematurity and maturational vestibular weakness.[21]

GJB2-related deafness occasionally cosegregates with a skin abnormality characterized by hyperkeratosis of the palms and soles, often with peeling, known as palmoplantar keratoderma (PPK), but this occurrence is rare and is associated only with specific *GJB2* allele variants.[23] Vohwinkel syndrome (VS), a specific form of PPK and deafness caused by the D66H mutation, has the additional component of autoamputation secondary to bandlike circumferential constrictions of the digits.[24] However, as a general rule, comorbid conditions are uncommon with *GJB2*-related deafness, and vision, intelligence, electrocardiography, and thyroid function are normal.[2,11,25,26]

Genetic screening of *GJB2* to establish a diagnosis of DFNB1 does have important limitations. First among these are the limitations in genetic testing itself.[27] Although *GJB2* is a small gene with only a single coding exon, the more than 80 different allele variants associated with ARNSD are scattered throughout the gene, making mutation screening of the entire coding sequence essential. In spite of this thorough approach, the identification of a single deafness-causing allele variant is not uncommon, implying the presence of a "missed" mutation in a noncoding region or coincidental carrier status in a person with deafness of another etiology.

A second limitation is limited genotype-phenotype correlation for predicting the degree of deafness. Among persons with Cx26 deafness, the degree of deafness can vary from mild to profound (mild [<40 dB], 1.7%; moderate [40–55 dB], 10.3%; moderately severe [56–70 dB], 7.8%; severe [71–90 dB], 30.2%; profound [>90 dB], 50.0%), even among persons with the same mutations.[2,11,19,28,29] Typically, the audiogram has a downsloping or flat pattern.[21] Selective midfrequency loss is rare,[28] and selective low-frequency loss has not been described. Symmetry between ears is normal, although one fourth of individuals have intra-aural differences of up to 20 dB.[11,20] The loss tends to be stable, with neither improvement in hearing nor fluctuation in hearing level over the long term.[21]

Available Assays

A number of mutation detection strategies can be used to screen *GJB2* for nucleotide changes, including restriction enzyme digestion, allele-specific polymerase chain reaction (AS-PCR) analysis, single-strand conformation polymorphism (SSCP) analysis, heteroduplex analysis (HA), denaturing high-performance liquid chromatography (DHPLC), and direct sequencing. Of these methods, SSCP and HA were among the first methods to be used to detect genetic polymorphisms and remain very popular because of their simplicity. However, the gold standard for establishing the identity of unknown nucleotide sequences is direct sequencing.

Interpretation of Test Results

The theoretical sensitivity of *GJB2* mutation screening based solely on AS-PCR detection of the 35delG mutation (defined as the subportion of the population with *GJB2*-related deafness and the 35delG mutation divided by the total population with *GJB2*-related deafness) has been calculated at 96.9% (range, 95.4% to 98.0%).[2] Calculated specificity (defined as the subportion of the population with *GJB2*-unrelated deafness not coincidentally carrying the 35delG mutation divided by the population with *GJB2*-unrelated deafness) is equally high at 97.4% (range, 97.0% to 98.0%). The observed sensitivity and specificity of 94% and 97%, respectively, are comparable to these values.[2]

These calculations assume that the population is randomly mating with respect to *GJB2*. The existence of population substructure, particularly endogamous subpopulations, results in a decreased proportion of heterozygotes (Wahlund's effect), with an overestimation of sensitivity for the population as a whole. Other assumptions made in these calculations include complete penetrance, lack of ascertainment bias (i.e., equal referral rates regardless of genotype), and negligible heterozygote selection advantage, spontaneous mutation rate, and migration effects. Deviation of the actual population from Hardy-Weinberg equilibrium due to these factors is likely to be minimal and does not affect the order of magnitude of the figures obtained, with the possible exception of assortative mating among the deaf.[2]

This mutation screening strategy of 35 delG mutation testing, however, misses *GJB2*-related deafness caused by non-35delG allele variants. More comprehensive mutation screens are based on DHPLC or direct sequencing. We have tested the sensitivity of DHPLC by screening a panel of 55 individuals carrying 52 combinations of 48 distinct *GJB2* sequence variants. Amplicons were analyzed at 62°C, 60°C, and 58°C to increase DHPLC detection efficiency, since all mutations cannot be detected at 62°C (L90P and I230T are not detected at 62°C). DHPLC wave profiles were analyzed for differences in shape and retention time compared to wild-type samples in four separate runs to test detection repeatability. On this basis, we have found DHPLC more sensitive at detecting *GJB2* allele variants than SSCP analysis, the comparative detection rates being 98.1% and 82.3%, respectively. Other authors have reported the sensitivity of DHPLC to range from 95% to 100%.[30–33]

Laboratory Issues

Determination of *GJB2*-related deafness is dependent on the identification of mutations in the DNA of affected individuals. Mutation screening of only exon 2 of *GJB2* by any technique is incomplete because there are two common noncoding, noncomplementary DFNB1-causing mutations that must be considered in persons heterozygous for a known *GJB2* deafness-causing allele variant. These mutations are the intron 1 splice donor mutation (IVS1+1G→A) and the large 5′ 342 kilobase (kb) deletion that includes a portion of *GJB6* and an additional upstream sequence (Δ[*GJB6*-D13S1830]). Based on the relative frequency of *GJB2* allele variants in the general population, the frequency of noncoding *GJB2* mutations associated with deafness at the DFNB1 locus, and phenotype-genotype correlations,[2] the existence of at least one additional mutation associated with the DFNB1 phenotype that is outside the coding region of *GJB2* is predicted.

For mutation screening by DHPLC, we use the acetonitrile gradient and partial denaturing temperature predicted by Wavemaker software, but add analysis 2°C above and below the predicted temperature to detect all possible mutations. To increase column life, cleanup and equilibration durations are extended and hot washing of the column is performed every 200 injections. DHPLC standards are run every 200 injections to confirm column reliability, since the ability of the column to detect standards is directly related to its ability to detect sequence variants in *GJB2* and other genes of interest. Water quality is checked by testing resistivity and total organic content. The purity of PCR products is verified by analyzing a sample at 50°C prior to analysis at partial denaturing conditions on the DHPLC.

PENDRED SYNDROME AND DFNBH (*SLC26A4*)

Molecular Basis of Disease

Mutations in *SLC26A4* (formerly known as *PDS*) cause Pendred syndrome (PS; OMIM #274600), an autosomal recessive disorder characterized by sensorineural deafness and goiter.[34] The deafness is congenital and associated with temporal bone abnormalities that range from isolated enlargement of the vestibular aqueduct (dilated vestibular aqueduct, DVA) to Mondini dysplasia, a more complex malformation that also includes cochlear hypoplasia, an anomaly in which the normal cochlear spiral of 2.5 turns is replaced by a smaller coil of 1.5 turns. Both DVA and Mondini dysplasia are easily recognized by computed tomography or magnetic resonance imaging.[35]

The thyromegaly in PS is the result of multinodular goitrous changes in the thyroid gland that develop in the teenage years,[36] although affected persons typically remain euthyroid with elevated serum thyroglobulin levels. The perchlorate discharge test is often abnormal. In this test, a person is given radiolabeled iodide and its localization to the thyroid is measured. Potassium perchlorate, a competitive inhibitor of iodide transport into the thyroid, then is administered. Normally, the amount of iodide in the thyroid will remain stable, reflecting rapid oxidation of iodide to iodine as it is incorporated into thyroglobulin. However, in a person with PS, iodide transport into the thyrocyte is delayed and so when perchlorate is

administered and blocks the sodium-iodide symporter, cytoplasmic iodide leaks back into the bloodstream. This back leakage is quantifiable as a change in thyroid radioactivity, with a positive result reflecting a drop in radioactivity of greater than 10%.[37]

In addition to PS, mutations in *SLC26A4* cause DFNB4 (OMIM #600791), a type of autosomal recessive nonsyndromic deafness in which, by definition, affected persons do not have thyromegaly.[38] No other physical abnormalities cosegregate with the deafness, although abnormal inner ear development, and in particular DVA, can be documented by temporal bone imaging. Together, DFNB4 and PS are estimated to account for 1% to 8% of congenital deafness.

Functional studies suggest that some of the observed differences between PS and DFNB4 are due to the degree of residual function of the encoded protein pendrin. While the function of pendrin is not fully determined, by homology it is thought to function in the transport of negatively charged particles (particularly chloride, iodide, and bicarbonate) across cell membranes. Mutations that abolish all transport function are more likely to be associated with the PS phenotype, while retained minimal transport ability appears to prevent thyroid dysfunction, although sensorineural deafness and temporal bone anomalies still occur, as in DFNB4.[39]

Many clinical studies have demonstrated intrafamilial variability, at times making the distinction between DFNB4 and PS difficult. The perchlorate discharge test is not a reliable test to resolve phenotypic ambiguities and is not consistently positive.[36] For example, in one family with two affected siblings, one child demonstrated the classic features of PS with severe-to-profound deafness, goiter, and a positive perchlorate discharge test, but the other child had only mild sensorineural deafness and no goiter.[40] In another study in which six individuals had confirmed PS, only three had a positive perchlorate washout of greater than10%.[41]

In a large clinical study of *SLC26A4* mutations in relation to temporal bone abnormalities, deafness-causing mutations were demonstrated in approximately 80% of multiplex families segregating DVA or Mondini dysplasia but in only 30% of simplex families.[42] These data suggest that allele variants of *SLC26A4* are a major genetic cause of these temporal bone abnormalities.

Since thyroid enlargement is an unreliable clinical indicator of disease and the perchlorate discharge test can be ambiguous, several investigators have recommended genetic testing of *SLC26A4* to establish a clinical diagnosis.[36,42] To date, 62 mutations have been reported in a total of 116 families.[43] Most of these mutations have been reported in only single families; however, 15 mutations are more common, and four (L236P, IVS8+1G→A, E384G, and T416P) account for approximately 60% of the total PS genetic load.[42] This broad spectrum of deafness-causing allele variants means that mutation screening of *SLC26A4* must include an analysis of all 20 protein-encoding exons (2–21) in addition to the splice donor site of exon 1.

Clinical Utility of Testing

Clinical data suggest that the major genetic cause of DVA and Mondini dysplasia is mutations in *SLC6A4*.[42] Because simplex cases include both genetic and nongenetic causes of DVA and Mondini dysplasia, a corollary is that most sporadic cases of DVA (~80%) and many sporadic cases of Mondini dysplasia (~40%) are not genetic and therefore are unlikely to recur in a family. This fact can be used to modify recurrence risks.

There is no concordance between specific *SLC26A4* allele variants and audiogram configuration,[42] although some mutations may be associated more frequently with specific temporal bone anomalies. For example, Masmoudi et al. studied two families segregating for L445W and found that while affected persons showed phenotypic variability with respect to thyroid disease, the temporal bone imaging revealed only DVA.[44]

Available Assays

A number of mutation detection strategies can be used to screen *SLC26A4* for nucleotide changes, including SSCP, HA, DHPLC, and direct sequencing. Because of its large size, high-throughput mutation screening of *SLC26A4* is challenging. The application of SSCP and HA is relatively insensitive and unnecessarily laborious, while direct sequencing is expensive. These constraints have made DHPLC an attractive alternative for mutation screening of *SLC26A4*.

Interpretation of Test Results

To compare the sensitivity of SSCP and DHPLC for detection of allele variants of *SLC26A4*, Prasad et al. screened a panel of 55 individuals segregating 41 different sequence-verified coding mutations. All 41 allele variants of *SLC26A4* were identified by DHPLC by their elution profile, for a detection rate of 100%. Nineteen mutations were detected at all three partial denaturing temperatures, ten mutations were detected at two of three temperatures, and 12 mutations were detected at only one temperature. Of the four common mutations, L236P and E384G showed discrete elution profiles at all three temperatures, but T416P and IVS8+1G→A were detected at only two and one temperatures, respectively. Mutations were tested multiple times from different samples to confirm test-retest reliability. Elution profiles of the four most common *SLC26A4* mutant alleles were distinct and easily could be differentiated from one another.

SSCP detected 26 (63%) of these same allele variants. Of the missed mutations, five (V138F, G209V, FS400, G672E, H723R) have been reported in more than one family. Three of the most common mutations (L236P, IVS8+1G→A, T416P) were detected by SSCP, although detection of L236P and IVS8+1G→A was not consistent.[45]

The finding that DHPLC sensitivity for detecting *SLC26A4* allele variants is 100% is similar to results reported by Taliani et al.[33] for DHPLC screen of *PROC*, a gene that encodes protein C, for variant identification. More than 200 different mutations in this gene are associated with an increased susceptibility to venous thromboembolism.[33] Other authors have reported the sensitivity of DHPLC to range from 95% to 100%.[30–32]

Laboratory Issues

To ensure a high level of accuracy and reliability and to optimize cost-effectiveness and turnaround time, a number of testing parameters must be considered. Amplicons should be maintained between 200 and 1,000 base pairs for accurate DHPLC analysis, intronic primers should be selected sufficiently far upstream and downstream of splice sites, and PCR conditions must be optimized for high-stringency exon amplification. Although the optimal acetonitrile gradient and partial denaturing temperature are determined using Wavemaker software, additional testing must be performed 2°C above and below the predicted temperature to detect all possible mutations.

While other investigators have been able to correlate DHPLC chromatogram profile with mutation type,[33] this correlation may be unreliable. The profile for a given *SLC26A4* allele variant differs from column to column and even in the same column, based on column life and buffer constitution. However, all heterozygote mutations are distinguished from wild-type samples. Because DHPLC does not distinguish homozygote allele variants due to the limitations inherent in HA, homozygotes can be detected by analysis following pooling of the unknown samples with sequence-verified wild-type DNA at a ratio of 2 : 1. By coupling additional automated instrumentation with DHPLC, high-throughput, accurate, reliable, and cost-effective mutation screening of persons with a Pendred syndrome/DNFB4 phenotype is possible.

Detection of only a single mutation is more common for testing of simplex families.[42,46] In three multiplex families segregating single mutations, Southern hybridization, and real-time PCR have failed to identify abnormalities in the "normal" allele, although all affected persons within a family had the same parental "normal" allele.

WOLFRAM SYNDROME AND DFNA6/14 (*WFS1*)

Molecular Basis of Disease

Mutations in *WFS1* cause autosomal dominant low-frequency sensorineural hearing impairment (LFSNHI) at the DFNA6/14 loci (OMIM #600965).[47,48] Deafness is bilaterally symmetrical and affects frequencies below 4,000 Hz; hearing is most impaired at the lowest frequencies, giving the DFNA6/14 audiogram an upsloping configuration.[49–52] DFNA1 also is characterized by LFSNHI, but in contrast to DFNA6/14 deafness, DFNA1 deafness is rapidly progressive and ultimately affects all frequencies.[53] Progression of DFNA6/14 deafness is minimal, although with aging the consequences of presbycusis result in flattening of the audiogram.

Mutations in *WFS1* also cause Wolfram syndrome, an autosomal recessive disease characterized by diabetes insipidus, diabetes mellitus, optic atrophy, and deafness, giving rise to the acronym DIDMOAD for this disease.[54,55] Minimal diagnostic criteria are diabetes mellitus and optic atrophy, and in addition to diabetes insipidus and sensorineural deafness, peripheral neuropathy, urinary-tract atony, and psychiatric illness can occur. Remarkably, the hearing loss in DIDMOAD syndrome is in the high frequencies.[56,57]

The observed phenotypic differences between DIDMOAD and DFNA6/14 appear to have a genotypic correlate. Sixty-five percent of persons with DIDMOAD carry inactivating mutations in *WFS1*, suggesting that loss of function of the encoded protein is causally related to Wolfram syndrome; most of these mutations lie in predicted transmembrane domains. In contrast, all disease-causing DFNA6/14 allele variants have missense mutations, and with one exception these amino acid changes are in the fifth intracellular domain of WFS1.[58,59] The protein lacks significant homology to published DNA or protein sequences, but secondary structure predictions suggest that it is has nine helical transmembrane segments. Biochemical studies suggest that wolframin is an endoglycosidase H-sensitive membrane glycoprotein predominantly located in the endoplasmic reticulum (ER).[60] Although the function of wolframin within the inner ear is unknown, it may play a role in the canalicular reticulum, a specialized ER that maintains intracellular ion homeostasis. Functional studies are necessary to test this hypothesis and to determine how different mutations in *WFS1* give rise to different phenotypes.

Clinical Utility of Testing

WFS1 contains eight exons (exon 1 is noncoding), encompasses 33.4 kb of genomic DNA, and transcribes a messenger RNA (mRNA) of 3.6 kb that encodes an 890 amino acid protein with a predicted molecular mass of 100 kilodaltons (kDa).[54,55] Mutations in *WFS1* are a major cause of LFSNHI in families demonstrating an autosomal dominant segregation pattern; however, mutation screening of *WFS1* in simplex cases is unlikely to identify abnormal allele variants.

Available Assays

A number of mutation detection strategies can be used to screen *WFS1* for nucleotide changes, including DHPLC and direct sequencing. A *WFS1* mutation database is available

to track the latest information on *WFS1* mutations in DIDMOAD and LFSNHI. This database lists all known mutations and polymorphisms with corresponding references.[61] Electronic submission of new mutations and polymorphisms is encouraged.

Interpretation of Test Results

Only noninactivating mutations are found in LFSNHI, and nearly all are located in the C-terminal region of the protein, suggesting a dominant-negative effect.[58] Although no clear-cut genotype-phenotype correlation has been drawn for DIDMOAD, homozygosity or compound heterozygosity for missense mutations is rarely found, and when it does occur, affected persons have a relatively mild phenotype that includes optic atrophy, diabetes mellitus, and sometimes hearing impairment, but excludes diabetes insipidus and other clinical findings. Persons carrying two deletions, insertions, nonsense mutations, or splice site mutations rarely have a mild phenotype.[59]

The disease spectrum in DIDMOAD has focused attention on the role of *WFS1* in diabetes mellitus and psychiatric diseases. The R456H, H611R, and I720V allele variants have been significantly correlated with type 1 diabetes mellitus in the Japanese, with a marginally significant association between R456H and type 2 diabetes mellitus.[62] Other case-control studies from the United Kingdom and Spain appear to confirm the involvement of *WFS1* in the pathogenesis of type 2 diabetes mellitus.[63,64] The exact role of *WFS1* in the etiology of diabetes is not known.

Laboratory Issues

Over 70 coding allele variants of *WFS1* have been reported, including synonymous and nonsynonymous changes.[61] Many of these changes have been found in persons with DIDMOAD and DFNA6/14, and cosegregate with known disease-causing mutations. Those that have been detected in controls are likely to be benign polymorphisms, but the effect of other mutations (K193Q, L432V, L499F, G576S, A559T, A671V, A684V, R708C, R818C, D866N, V871M) on disease phenotype is more difficult to judge, as they have been described both as disease-causing mutations and as polymorphisms.[59]

BRANCHIO-OTO-RENAL-SYNDROME (*EYA1*)

Molecular Basis of Disease

Mutations in *EYA1* cause branchio-oto-renal (BOR) syndrome (OMIM #113650).[65–69] Disease prevalence is estimated at 1 in 40,000 in the general population, and the syndrome is reported to occur in about 2% of profoundly deaf children.[70] Clinical expression is highly variable within and among families, but typical manifestations include branchial arch anomalies (preauricular pits, branchial fistulae, and pinnae abnormalities), hearing loss (conductive, sensorineural, or mixed), and renal hypoplasia.[71]

Phenotypic features that occur in more than 20% of affected persons are classified as major. Hearing loss and preauricular pits are most prevalent, affecting approximately 90% and 80% of individuals, respectively. Branchial cleft fistulae (~50%), lop-ear deformity (~35%), and stenotic external auditory canals (~30%) also are common. Temporal bone abnormalities can be identified in most individuals with hearing impairment examined by computed tomography, and renal anomalies are identified in approximately 65% of individuals examined by ultrasound or excretory urography.[71]

Hearing loss is mixed (~50%), conductive (~25%), or sensorineural (~25%), and ranges from mild to profound, but is most commonly severe (~35%), and is progressive in approximately 25% of affected persons. Temporal bone abnormalities include stenosis and atresia of the external auditory canal, malformation, malposition, dislocation or fixation of the ossicles, and reduction in size or malformation of the middle ear cavity. In the inner ear, the most common anomaly is cochlear hypoplasia. Enlargement of the cochlear and vestibular aqueducts and hypoplasia of the lateral semicircular canal also are found. Major renal anomalies include agenesis (most common), hypoplasia, and dysplasia. Calyceal diverticula, ureteral pelvic junction obstruction, hydronephrosis, pelviectasis, calyectasis, and vesico-ureteral reflux also are seen.[71]

In some families, anticipation may appear to be present; however, a study of seven three-generational families assessed for anticipation yielded conflicting results. In four of these families, the degree of hearing loss showed anticipation, but in the remaining three, a generational loss did not occur.[71] With respect to renal disease, generational progression was present in three families, but in one family, the reverse trend was found.[71] There is also no evidence for a parent-of-origin effect. Marked renal defects have been reported in six live-born offspring (including bilateral renal agenesis in three individuals) who had affected fathers and in 4 live-born offspring of affected mothers.[72–77] An excess of unexplained fetal deaths, presumably a consequence of bilateral renal agenesis, occurred in all of these families.

Clinical Utility of Testing

EYA1 contains 16 exons, encompasses 164.8 kb of genomic DNA, and transcribes an mRNA of 1.7 kb that encodes a 559 amino acid protein with a predicted molecular mass of 61.2 kDa. Mutations in *EYA1* are a major cause of hearing loss in families segregating a BOR phenotype; however, mutation screening of *EYA1* in simplex cases is unlikely to identify abnormal allele variants.[43]

Available Assays

Mutation screening of *EYA1* currently is available by SSCP analysis, with sequencing of exons in which band shifts are identified. An *EYA1* mutation database is available to track the latest information on *EYA1* mutations in BOR syndrome. This database lists all known mutations with corresponding references. Electronic submission of new mutations and polymorphisms is encouraged.[43]

Interpretation of Test Results

Initial reports on BOR syndrome described a patient with a complex cytogenetic rearrangement on chromosome 8q[78,79] and another patient with 8q12.2-q21 deletion,[80] indicating that complex genomic rearrangements can cause this phenotype. In the first two mutation analyses of *EYA1*, three large deletions are described.[65,66] Current estimates suggest that about one fifth of *EYA1* mutations resulting in BOR syndrome represent complex genomic rearrangements, perhaps indicating that the *EYA1* region is unstable.[81] These rearrangements cannot be detected by commonly used mutation screening procedures, making it impossible to use a single approach for mutation screening of *EYA1*.

Laboratory Issues

Although numerous reports have confirmed that alterations in *EYA1* cause BOR syndrome,[66–70] in many of these reports only 20% of affected patients have confirmed *EYA1* mutations.[81] To explain this low mutation detection rate, some investigators have hypothesized that mutations in another gene tightly linked to *EYA1* also cause BOR syndrome. However, using Southern analysis, other investigators have been able to detect mutations in families linked to chromosome 8, in which SSCP analysis and direct sequencing fail to detect *EYA1* mutations.[81] This finding underscores the major drawback of many PCR-based mutation detection procedures in the study of an autosomal dominant disorder, the inevitable amplification of the normal allele that can mask deleted or disrupted exons.

Conclusion

With continued advances in our understanding of the molecular biology of hearing and deafness, the clinical management of deafness will become more sophisticated. Molecular genetic testing will be used to unequivocally diagnose many different forms of inherited deafness, providing prognostic information for patients and their families. Novel habilitation options will be developed that will be applicable to select persons with specific types of genetic deafness. At the forefront of these advances will be the ability to offer precise genetic testing that is rapid, robust, and cost-effective.

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