EYA1 and SIX1 Gene Analysis in Branchiootorenal (BOR)/
Branchiootic (BO) Syndrome

Also known as: Branchiootorenal Dysplasia, Melnick-Fraser Syndrome, Eyes absent homolog 1

Mendelian Inheritance in Man Number: 113650 (Branchiootorenal syndrome 1, BOR1); 601653 (Eyes absent homolog 1, EYA1); 601205 (Homeobox protein SIX1).

Clinical features:
BOR (Branchiootorenal) syndrome, which clinically overlaps with branchiootic syndrome, is characterized by multiple malformations clinically diagnosed by the following major criteria: second branchial arch anomalies, deafness, preauricular pits, auricular deformities and renal anomalies (ranging from mild to severe or complete absence of kidneys). Minor criteria include: external auditory canal anomalies, middle or inner ear anomalies, preauricular tags and others. To be diagnosed there must be 2 affected individuals in the family; or the individual must display 3 or more of the major criteria, or 2 major and 2 minor criteria. Both reduced penetrance and variable expressivity have been observed. The estimated prevalence of BOR syndrome is 1:40,000 in the general population and ~2% among profoundly deaf children. Hearing impairment can be mild to severe and can be conductive, sensorineural or mixed.2

Inheritance pattern:
Autosomal Dominant. Approximately 10% of cases are caused by a de novo mutation.

Reasons for referral:
1. Confirmation of a clinical diagnosis
2. Differential diagnosis between other branchio and/or oto multiple malformation syndromes
3. Genetic counseling and risk assessment
4. Prenatal diagnosis

Test method:
Analysis is performed by bi-directional sequencing of all coding exons (exons 3-18) and splice sites of the EYA1 gene. Concurrently, targeted array CGH analysis with exon-level resolution (ExonArrayDx) is performed to evaluate for a deletion or duplication of one or more exons of this gene. Additionally, bi-directional sequencing of the coding regions and splice sites of the SIX1 gene (exons 1 and 2) is available. Mutations/deletions/duplications found in the first person of a family to be tested are confirmed by repeat analysis using sequencing, restriction fragment analysis, qPCR or another appropriate method.

Mutation spectrum:
More than 100 mutations in the EYA1 gene have been associated with BOR Syndrome, including nonsense, missense, splice site mutations, small deletions and insertions. Approximately 10-20% of patients with BOR are found to have large deletions of one or more exons, the entire gene, or chromosomal rearrangement involving the EYA1 gene.1,2,6
There are very few published cases of BOR/BO syndrome due to mutation in the SIX1 gene. Most mutations are missense changes affecting the homeodomain region, which is essential for specific Six1-DNA binding.4,5

Test sensitivity:
Up to 82% of patients meeting the diagnostic criteria listed above will have an EYA1 mutation detected by combined full gene sequencing and deletion/duplication testing.1,6,7 Two studies found that approximately 80% of mutations can be identified by sequence analysis of the coding sequence of EYA1, while the remaining 20% represent large
deletions or chromosomal rearrangements.1,6 Deletions/duplications of one or more exons can be detected by targeted array CGH analysis with exon-level resolution (ExonArrayDx). A balanced chromosomal rearrangement involving the EYA1 gene would be missed by our methods. The clinical sensitivity of SIX1 testing has not been established.

Specimen Requirements and Shipping/Handling:

- **Blood**: A single tube with 1-5 mL whole blood in EDTA. Ship overnight at ambient temperature, using a cool pack in hot weather. Specimens may be refrigerated for 7 days prior to shipping.
- **Buccal Brushes**: As an alternative to blood, **for SIX1 sequencing only, EYA1 testing requires submission of a venous blood sample.** Use a GeneDx buccal kit (others not accepted). Submit by mail. Buccal brushes are not accepted on children under 6 months of age.
- **Prenatal Diagnosis**: For prenatal testing for a known mutation in the SIX1 gene, please refer to the specimen requirements table on our website at: [http://www.genedx.com/test-catalog/prenatal/](http://www.genedx.com/test-catalog/prenatal/). Ship specimen overnight at ambient temperature, using a cool pack in hot weather.

Required Forms:

- Sample Submission (Requisition) Form – complete all pages, including
- Payment Options Form or Institutional Billing Instructions

References: