

## Prenatal testing for the POR Gene in Antley-Bixler syndrome/P450 Oxidoreductase Deficiency

**Disorder also known as:** POR deficiency; Congenital adrenal hyperplasia with combined P450C17 and P450C21 deficiencies

### Clinical Features:

**Children and Newborns:** Cytochrome P450 oxidoreductase (POR) deficiency is a disorder of steroidogenesis associated with a broad range of clinical presentations. Steroid abnormalities occur in all patients, consistent with a form of congenital adrenal hyperplasia (CAH) causing deficiencies of both 21-hydroxylase and 17-hydroxylase/17,20-lyase; therefore, some patients with POR deficiency may have an abnormal newborn screen for 21-hydroxylase deficiency. Like classical CAH, the steroid abnormalities may lead to cortisol deficiency, which in some cases can be life-threatening without treatment<sup>1</sup>. Disordered sex development can be observed in both males and females, and ambiguous genitalia is a common finding. At the severe end of the spectrum, patients may also have skeletal and craniofacial findings consistent with Antley-Bixler syndrome, including craniosynostosis, brachycephaly, severe midface hypoplasia, radiohumeral synostosis, and multiple joint contractures.

**Prenatal Ultrasound and Biochemical Findings:** Ultrasound evaluation in affected pregnancies may identify fixed flexion of elbows, bowing of long bones, hypoplastic midface, depressed nasal bridge, brachycephaly, rocker-bottom feet, and ambiguous genitalia<sup>2,3</sup>. The fixed flexion of elbows is an important finding<sup>3</sup>. Ultrasound examination may be normal in affected fetuses; therefore, pregnancies at risk for POR deficiency due to a positive family history can be offered molecular testing regardless of ultrasound findings, if desired. Affected pregnancies are reported to have very low or undetectable levels of unconjugated estriol in maternal serum screening at 15-20 week's gestation. Additionally, maternal virilization during pregnancy with an affected fetus can be observed with onset in the second trimester<sup>4,5</sup>.

### Genetics:

Autosomal Recessive. The POR gene is located on chromosome 7q11.2. It is approximately 32-kb and consists of 15 coding exons. It is ubiquitously expressed and produces an 82-kDA membrane-bound protein that plays an important role in steroid and cholesterol synthesis. The protein has two distinct domains, one containing both an NADPH-binding site and an FAD binding site and the other containing the FMN domain. The POR protein binds NADPH and accepts a pair of electrons through its FAD component. The electrons are transferred to the FMN moiety and then are distributed to other cytochrome P450 enzymes, including CYP17A1, CYP21A2, CYP51A1, and CYP19A1.

## Test Methods:

Using genomic DNA extracted from the submitted specimen, the complete coding regions and splice site junctions of the genes tested are enriched using a proprietary targeted capture system developed by GeneDx for next-generation sequencing with CNV calling (NGS-CNV). The enriched targets are simultaneously sequenced with paired-end reads on an Illumina platform. Bi-directional sequence reads are assembled and aligned to reference sequences based on NCBI RefSeq transcripts and human genome build GRCh37/UCSC hg19. After gene specific filtering, data are analyzed to identify sequence variants and most deletions and duplications involving coding exons; however, technical limitations and inherent sequence properties effectively reduce this resolution for some genes. Alternative sequencing or copy number detection methods are used to analyze or confirm regions with inadequate sequence or copy number data by NGS. Reportable variants include pathogenic variants, likely pathogenic variants and variants of uncertain significance. Likely benign and benign variants, if present, are not routinely reported but are available upon request.

The technical sensitivity of sequencing is estimated to be >99% at detecting single nucleotide events. It will not reliably detect deletions greater than 20 base pairs, insertions or rearrangements greater than 10 base pairs, or low-level mosaicism. The copy number assessment methods used with this test cannot reliably detect copy number variants of less than 500 base pairs or mosaicism and cannot identify balanced chromosome aberrations. Assessment of exon-level copy number events is dependent on the inherent sequence properties of the targeted regions, including shared homology and exon size. Gene specific exclusions for exon-level deletion/duplication testing for this panel are: KIF7 gene, only whole gene deletions or duplications may be detected.

Additionally, genotype analysis of maternal and fetal DNA for several polymorphic markers to test for maternal cell contamination will be performed. Therefore, in all prenatal cases, a maternal sample should accompany the fetal sample.

## Test Sensitivity:

POR sequencing is not well established at this time. An estimated 12% of patients reported to date have only one identifiable variant in the POR gene, indicating that some variants lie outside of the coding exons and would not be detected with current methodology<sup>6</sup>. Antley-Bixler syndrome is genetically heterogenous. Variants in the POR gene have been identified in patients with skeletal and craniofacial features of ABS who also have genital anomalies and/or disordered steroidogenesis, whereas variants in the FGFR2 gene have been identified in patients with a clinical diagnosis of ABS in the absence of genital or steroid anomalies<sup>7,8</sup>. In the largest study to date, 19/32 (59%) patients with a clinical diagnosis of ABS had at least one identifiable variant in the POR gene, including 15/15 (100%) with abnormal steroids and/or

genitalia<sup>7</sup>. The sensitivity of POR analysis in prenatal cases ascertained based on fetal ultrasound abnormalities is currently unknown.

## References:

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