PAH Gene Analysis in Phenylketonuria

DISORDER ALSO KNOWN AS
PKU; Phenylalanine hydroxylase deficiency, PAH deficiency; Hyperphenylalanemia; HPA; Mild hyperphenylalanemia

CLINICAL FEATURES
PAH deficiency is a well-characterized, treatable, biochemical disorder which results in dietary intolerance to the essential amino acid phenylalanine. It is the most common inborn error of amino acid metabolism in the Caucasian population with an average incidence of 1 in 10,000. PAH deficiency is a condition with a broad phenotypic spectrum that ranges from classic phenylketonuria (PKU) to mild hyperphenylalaninemia (HPA), depending on phenylalanine levels. Most individuals with untreated classic PKU exhibit severe irreversible intellectual disability. Microcephaly, epilepsy, behavioral problems, eczema, hypopigmentation, decreased myelin formation, and a musty urine odor may also be present. Untreated mild HPA may result in mild symptoms depending on the phenylalanine level.[1]

More rarely, individuals may have hyperphenylalaninemia due to a tetrahydrobiopterin (BH_4) deficiency. BH_4 deficiency is due to defects in the enzymes involved in the synthesis or regeneration of tetrahydrobiopterin (BH_4), a cofactor for phenylalanine hydroxylase enzyme. BH_4 deficient hyperphenylalaninemia is a genetically heterogeneous group of disorders caused by variants in several genes (GCH1, PTS, QDPR, PCBD1) of the BH_4 pathway.[2,3]

GENETICS
PAH deficiency is caused by variants in the PAH gene. The PAH gene encodes the enzyme phenylalanine hydroxylase. The primary route for phenylalanine metabolism is hydroxylation of phenylalanine to tyrosine catalyzed by phenylalanine hydroxylase; consequently a deficiency of this enzyme leads to an elevation of the plasma phenylalanine (phe) concentration (~1000 µmol/L). The presence of specific variants may be correlated with severity or with responsiveness to BH_4 therapy.[1] The PAH gene is located on chromosome 12q23.2 and has 13 coding exons.

INHERITANCE PATTERN
Autosomal Recessive

TEST METHODS
Using genomic DNA extracted from the submitted specimen, the complete coding regions and splice site junctions of the PAH gene 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 the reference sequence 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.

A gene panel to test for disorders of hyperphenylalaninemia and biopterin metabolism, which includes analysis of the PAH, PTS, GCH1, SPR, QDPR, PCBD1, and DNAJC12 genes, is also available at GeneDx

Variant Spectrum

To date, over 900 variants have been reported in the PAH gene in all 13 exons. The most common types of variants include missense, slice-site, and small deletions. Nonsense variants, regulatory variants, small insertions, and gross deletions/duplications have also been reported.[4] Common pathogenic variants in many populations have been reported.

Specific PAH genotype is the major predictor of metabolic phenotype. In patients with compound heterozygous variants and functional hemizygosity (null/missense paired alleles), disease severity in most cases is determined by the least severe of the two PAH variants. Additionally, it has been shown that two PAH variants of similar severity may render a milder phenotype than would be predicted by either variant alone.[1]

REFERENCES:

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