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]

Patients with hyperphenylalaninemia due to tetrahydrobiopterin (BH_4) deficiency will not be expected to have pathogenic variants in *PAH*. 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 genes (*GCH1, PTS, QDPR, PCBD1, SPR)* 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). Specific variants may determine phenotype and 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:
Analysis is performed by bi-directional sequencing of the coding regions (exons 1-13) and splice sites of the *PAH* gene. If sequencing identifies a variant on only one allele of the *PAH* gene, and if clinically indicated, reflex deletion/duplication testing (ExonArrayDx) will be performed at no additional charge to evaluate for a deletion/duplication of one or more exons

of this gene. Variants found in the first person of a family to be tested are confirmed by repeat analysis using sequencing, restriction fragment analysis, or another appropriate method.

Test Sensitivity:

Bi-directional sequencing of the entire coding region and intron-exon boundaries of the *PAH* gene by GeneDx will identify more than 95% of variants. Some variants, such as large deletions or variants in the promoter region will not be detected by this method. If both variants are not detected by sequencing, targeted array CGH analysis with exon-level resolution (ExonArrayDx) will be performed to evaluate for a deletion or duplication of one or more exons of the *PAH* gene. The methods used by GeneDx are expected to be greater than 99% sensitive at detecting variants identifiable by sequencing.

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 (i.e., mild PKU is 'dominant'). Additionally, it has been shown that two *PAH* variants of similar severity may render a milder phenotype than would be predicted by either variant acting alone.[1]

References:

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3. Ye et al. (2013) Journal Of Inherited Metabolic Disease 36 (5):893-901 (PMID: 23138986)
4. Stenson et al. (2014) Human Genetics 133 (1):1-9 (PMID: 24077912)