EGL Genetics | Eurofins Clinical Diagnostics
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Holocarboxylase Synthetase Deficiency: *HLCS* Gene Sequencing

Test Code: JW
Turnaround time: 4 weeks
CPT Codes: 81479 x1

Condition Description

Holocarboxylase Synthetase Deficiency (HLCS) is an autosomal recessive inborn error of biotin metabolism [1]. It is also called early-onset multiple carboxylase deficiency and is clinically and biochemically similar to the disorder late-onset multiple carboxylase deficiency, or biotinidase deficiency, a separate disorder caused by mutations in the biotinidase gene *BTD* (refer to the Biotinidase Deficiency test for more information) [2].

Biotin is an essential water-soluble vitamin that serves as a coenzyme for four carboxylases in humans (acetyl-CoA carboxylase, pyruvate carboxylase, propionyl-CoA carboxylase, and b-methylcrotonyl-CoA carboxylase) [3]. Its serum level depends on dietary biotin intake and the recycling of endogenous biotin. The normal function of a carboxylase protein requires establishment of a covalent bond with the cofactor biotin. HLCS establishes a covalent bond between a lysine residue in the apocarboxylase molecule and a biotin molecule and is therefore crucial in biotin recycling.

The age of onset is one of the distinguishing factors with HLCS typically presenting between birth and 3 months of age and biotinidase deficiency typically presenting after 3 months. The symptoms in these disorders are similar and clinical differentiation is often difficult. In untreated states, both are usually characterized by seizures, hypotonia, ataxia, developmental delay, vision problems, hearing loss, and cutaneous changes such as alopecia, skin rash, and candidiasis. With age, motor limb weakness, spastic paresis, and decreased visual acuity occur. Both HLCS and biotinidase deficiency are biotin-responsive and early recognition and biotin supplementation result in rapid clinical improvement [4-5]. Newborn screening allows early presymptomatic treatment that can prevent neurological deterioration [6].

Organic acid abnormalities are similar in HLCS and biotinidase deficiency and may be reported as consistent with multiple carboxylase deficiency on tandem mass spectrometry utilized in neonatal screening. Definitive enzyme determinations are required to distinguish between the two disorders [7]. Biotinidase activity is normal in serum of individuals with holocarboxylase synthetase deficiency; therefore, the enzymatic assay of biotinidase activity used in newborn screening is specific for biotinidase deficiency and does not identify children with holocarboxylase synthetase deficiency. Both biotinidase deficiency and holocarboxylase synthetase deficiency are characterized by deficient activities of the three mitochondrial carboxylases in peripheral blood leukocytes prior to biotin treatment. In both disorders, these activities increase to near-normal or normal after biotin treatment.

HLCS enzyme deficiency is caused by mutations in the *HLCS* gene located at the 21q22 [8]. About 30 mutations in the *HLCS* gene have been reported and a majority of them are missense and nonsense mutations with 5 polymorphisms described as well [9]. There is some evidence for genotype-phenotype correlation, e.g. the missense mutations L237P and L470S and the null mutations 780delG, 6556insA, and R665X were associated with reduced enzyme activity and earlier onset of the disease [9]. Gene sequence analysis is available to test for mutations in the *HLCS* gene (JW). For patients with mutations not identified by full gene sequencing, a separate deletion/duplication assay is available using a targeted CGH array (JX).

References:
1. Wolf B. Disorders of Biotin Metabolism, in: C.R. Scriver, A.L. Beaudet, W. Sly, D. Valle (Eds.), The Metabolic and Molecular Bases of Inherited Disease, McGraw-Hill, New York, 2001, pp. 3944-3947.
2. Sweetman L. Two forms of biotin-responsive multiple carboxylase deficiency. J Inherit Metab Dis 1981, 4:53-54.
3. Hart et al. Biochemical and immunological characterization of serum biotinidase in profound biotinidase deficiency. Am J Hum Genet 1992, 50:126-136.
4. Narisawa et al. Clinical and biochemical findings on a child with multiple biotin-responsive carboxylase deficiencies. J Inherit Metab Dis 1982, 5:67-68.
5. Packman et al. The neonatal form of biotin-responsive multiple carboxylase deficiency. J Pediat 1981, 99: 418-420.
6. Heard et al. Neonatal screening for biotinidase deficiency: results of a 1-year pilot Study. J Pediatr 1986, 108:40-46.
7. Bartlett et al. Enzyme studies in combined carboxylase deficiency. Ann NY Acad Sci 1985, 447:235-51.
8. Aoki et al. Identification and characterization of mutations in patients with holocarboxylase synthetase deficiency. Hum Genet 1999, 104:143-148.
9. Suzuki et al. Mutations in the holocarboxylase synthetase gene HLCS. Hum Mutat 2005, 26(4):285-90.
10. Morrone et al. Clinical Findings and Biochemical and Molecular Analysis of Four Patients With Holocarboxylase Synthetase Deficiency. Am J Med Genet 2002, 111:10-18.
11. Yang et al. Structure of human holocarboxylase synthetase gene and mutation spectrum of holocarboxylase synthetase deficiency. Hum Genet 2001, 109:526-534.

Indications

This test is indicated for:

- Confirmation of a clinical/biochemical diagnosis of HLCS deficiency
- Carrier testing in adults with a family history of HCLS deficiency

Methodology

PCR amplification of 14 exons contained in the HLCS gene is performed on patient genomic DNA. Direct sequencing of amplification products is performed in both the forward and reverse directions using automated fluorescence dideoxy sequencing methods. Patient gene sequences are compared to a normal reference sequence. Sequence variations are then classified as mutations, benign variants unrelated to disease, or variations of unknown clinical significance. Variants of unknown clinical significance may require further studies of the patient and/or family members. This assay does not interrogate the promoter region, deep intronic regions, or other regulatory elements. Large deletions are not detected by this analysis.

Detection

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The majority of patients with clinical and biochemical diagnosis of HLCS deficiency will have an abnormal DNA test.
Clinical Sensitivity: 18/18 mutations identified in 9 patients [8]; 6/8 mutations identified in 4 patients [10], 18/18 mutations identified in 9 patients [11].
Analytical Sensitivity: ~99%.
Results of molecular analysis must be interpreted in the context of the patient's clinical and/or biochemical phenotype.

Specimen Requirements

Submit only 1 of the following specimen types

Type: DNA, Isolated

Specimen Requirements:
Microtainer
8µg
Isolation using the Perkin Elmer™Chemagen™ Chemagen™ Automated Extraction method or Qiagen™ Puregene kit for DNA extraction is recommended.

Specimen Collection and Shipping:
Refrigerate until time of shipment in 100 ng/µL in TE buffer. Ship sample at room temperature with overnight delivery.

Type: Saliva

Specimen Requirements:
Oragene™ Saliva Collection Kit
Orangene™ Saliva Collection Kit used according to manufacturer instructions. Please contact EGL for a Saliva Collection Kit for patients that cannot provide a blood sample.

Specimen Collection and Shipping:
Please do not refrigerate or freeze saliva sample. Please store and ship at room temperature.

Type: Whole Blood (EDTA)

Specimen Requirements:
EDTA (Purple Top)
Infants and Young Children (2 years of age to 10 years old: 3-5 ml
Older Children & Adults: 5-10 ml
Autopsy: 2-3 ml unclotted cord or cardiac blood

Specimen Collection and Shipping:
Ship sample at room temperature for receipt at EGL within 72 hours of collection. Do not freeze.

Special Instructions

Laboratory, please submit a copy of the sequencing report with the diagnostic biochemical test results with the sample. Sequence analysis is required before deletion/duplication analysis by targeted CGH array. If sequencing is performed outside of Emory Genetics test requisition.

Related Tests

- Urine Organic Acids (OA), and Plasma Acylcarnitine Profile (AR) are used in the diagnoses of a patient with HLCS deficiency
- Biotinidase Assay (BX) may also be used in some instances to aid in diagnosis of HLCS deficiency
- Custom Diagnostic Mutation Analysis (KM) is available to family members if mutations are identified by sequencing.
- Deletion/Duplication Assay is available separately for individuals where mutations are not identified by sequence analysis. Refer to the test requisition or contact the laboratory for more information.
- Prenatal testing is available for known familial mutations only. Please call the Laboratory Genetic Counselor before collecting a fetal sample.

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