EGL Genetics | Eurofins Clinical Diagnostics
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Ketothiolase Deficiency: *ACAT1* Gene Sequencing

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

Condition Description

Beta-Ketothiolase deficiency (BKTD) is an autosomal recessive inborn error of ketone body and isoleucine metabolism [1]. Clinical manifestations of BKTD include intermittent episodes of severe ketoacidosis, usually with normoglycemia or hyperglycemia that can result in hyperventilation, dehydration, lethargy, coma, and death. Episodes are usually associated with severe vomiting and are triggered by infections or other illnesses. Therapy consists of mild protein restriction to limit the intake of isoleucine, avoidance of fasting, supplementation with carnitine, avoidance of prolonged fasting, and prompt treatment of illnesses that can precipitate acute attacks. The outcome of BKTD is favorable with early diagnosis, dietary therapy, and appropriate treatment of ketoacidosis. BKTD is caused by deficiency of enzyme 3-ketothiolase (also called mitochondrial acetoacetyl-CoA thiolase or T2). Analysis of urine organic acids during acute episodes reveals high excretion of 2-methyl-3-hydroxybutyrate, 2-methylacetoacetate, and tiglylglycine with large amounts of 3- hydroxy-butyrate and acetoacetate [2]. Analysis of plasma acylcarnitines shows increased concentrations of C5OH (2-methyl-3-hydroxybutyryl carnitine) and C5:1 (tiglyl carnitine). 3-ketothiolase is encoded by the *ACAT1* gene (11q22) which has been found to have heterogenous mutations in patients with BKTD [3-5]. Although no definitive correlation between phenotype and genotype has been identified, differences in the biochemical profiles under stable conditions between the groups with different mutations have been reported [6]. Newborn screening by tandem mass spectrometry can identify infants with BKTD caused by severe mutations, but may miss infants with the "milder" mutations. Gene sequence analysis is available to test for mutations in *ACAT1* gene (JE). For patients with mutations not identified by full gene sequencing, a separate deletion/duplication assay is available using a targeted CGH array (JF).

References:
1. Mitchell and Fukao. Inborn Errors of Ketone Body 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. 2344-2347.
2. Pasquali et al. Biochemical Findings in Common Inborn Errors of Metabolism. Am J Med Genet C 2006, 142C:64-76.
3. Mrazova et al. Two novel mutations in mitochondrial acetoacetyl-CoA thiolase deficiency. J Inherit Metab Dis 2005, 28:235-236.
4. Fukao et al. Characterization of six mutations in five Spanish patients with mitochondrial acetoacetyl-CoA thiolase deficiency: Effects of amino acid substitutions on tertiary structure. Mol Genet Metab 2002, 75:235-243. 5. Fukao et al. Single base substitutions at the initiator codon in the mitochondrial acetoacetyl-CoA thiolase (ACAT1/T2) gene result in production of varying amounts of wild-type T2 polypeptide. Hum Mutat 2003, 21(6):587-92.
6. Fukao et al. The clinical phenotype and outcome of mitochondrial acetoacetyl-CoA thiolase deficiency (beta-ketothiolase or T2 deficiency) in 26 enzymatically proved and mutation defined patients. Mol Genet Metab 2001, 72:109-114.
7. Sakurai et al. Kinetic and expression analyses of seven novel mutations in mitochondrial acetoacetyl-CoA thiolase (T2): identification of a Km mutant and an analysis of the mutational sites in the structure. Mol Genet Metab 2007, 90(4):370-378.
8. Zhang et al. Mitochondrial Acetoacetyl-CoA Thiolase (T2) Deficiency: T2-Deficient Patients with "Mild" Mutation(s) Were Previously Misinterpreted as Normal by the Coupled Assay with Tiglyl-CoA. Ped Res 2004, 56(1):60-64.

Genes

ACAT1

Indications

This test is indicated for:

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

Methodology

PCR amplification of 12 exons contained in the *ACAT1* 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

Clinical Sensitivity: 9/12 mutations identified in 6 patients [7], 6/10 mutations identified in 5 patients [8].
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: 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

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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.

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.

Special Instructions

Submit copies of 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 EGL Genetics, please submit a copy of the sequencing report with the test requisition.

Related Tests

- Plasma Amino Acid (AA) Analysis, Urine Organic Acids (OA), and Plasma Acylcarnitine Profiles (AR) are used in the diagnoses of a patient with BKTD. Urine Acylcarnitine and Acylglycine Profiles can also be helpful.
- Custom Diagnostic Mutation Analysis (KM) is available to family members if mutations are identified by sequencing.
- A 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.