

Trifunctional Protein Deficiency: *HADHA* & *HADHB* Gene Sequencing

Test Code: FZ

Turnaround time: 6 weeks

CPT Codes: 81479 x1

Condition Description

Trifunctional protein (TFP) deficiency is an autosomal recessive disorder of fatty acid oxidation [1-2]. Clinical presentation is variable, with three major presentations described. Newborns with the early-onset form can present with poor feeding, severe dilated cardiomyopathy, lactic acidosis, and Reye syndrome-like symptoms of hypoketotic hypoglycemia. A hepatic phenotype is described also described in infancy with recurrent hypoketotic hypoglycemia. Patients can also present in the first months of life with recurrent hypoketotic hypoglycemia and lethargy during illness or periods of fasting. In addition, a later onset neuromyopathic form is described in individuals with a later-onset, progressive peripheral neuropathy, muscle weakness, episodic myoglobinuria and high plasma CK concentrations. Developmental delay may be associated with a history of acute metabolic decompensation. A maternal presentation has been reported in women carrying a fetus with TFP deficiency characterized as either HELLP syndrome (hypertension, elevated liver enzymes, low platelets) and AFLP syndrome (acute fatty liver of pregnancy).

TFP is multi-enzyme complex composed of four alpha-subunits (*HADHA*), which contains the long-chain 2-enoyl-CoA hydratase and the long-chain L-3-hydroxyacyl-CoA dehydrogenase (LCHAD) domains, and four beta-subunits (*HADHB*), which contains the long-chain 3-ketoacyl-CoA thiolase (LKAT) domain [3-4]. Deficiency of these enzymes can result in diseases of the TFP complex with overlapping clinical symptoms: either the general TFP deficiency (described above) or isolated LCHAD deficiency (LCHADD). General TFP deficiency is defined by reduced activity of all three TFP enzymes. Isolated LCHAD deficiency is defined by reduced LCHAD activity resulting in accumulation of long-chain 3-hydroxy-fatty acids. The other two TFP enzymes might also be affected in LCHADD, but their activities are usually greater than 60% of normal (refer to the LCHAD test description for testing for LCHAD).

The TFP alpha- and beta-subunits are encoded by *HADHA* and *HADHB*, respectively, and genes both located in 2p23 [5]. An isolated LCHAD presentation is associated with the common alpha-subunit mutation 1528G>C (E474Q) that is located directly within the catalytic region of the LCHAD domain, though other genotype-phenotype correlations are limited [7-8]. TFP deficiency due to alpha-subunit mutations has been described more often than cases due to beta-subunit mutations [6]. Gene sequencing is available to test for mutations in the *HADHA* and *HADHB* genes (FZ). For patients with mutations not identified by full gene sequencing, a separate deletion/duplication assay is available using a targeted CGH array (GE).

References:

1. Roe and Ding. Mitochondrial Fatty Acid Oxidation Disorders, 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. 2308-2310.
2. Den Boer et al. Long-chain 3-hydroxyacyl-CoA dehydro-genase deficiency: clinical presentation and follow-up of 50 patients. *Pediatrics* 2002, 109:99-104.
3. Spiekerkoetter et al. Molecular and phenotypic heterogeneity in mitochondrial trifunctional protein deficiency due to beta-subunit mutations. *Hum Mutat* 2003, 21(6):598-607.
4. Choi et al. Identification of novel mutations of the *HADHA* and *HADHB* genes in patients with mitochondrial trifunctional protein deficiency. *Int J Mol Med* 2007, 19(1):81-7.
5. Yang et al. The genes for the [alpha] and [beta] subunits of the mitochondrial trifunctional protein are both located in the same region on human chromosome 2p23. *Genomics* 1996, 37:141-143.
6. Matern et al. Diagnosis of mitochondrial trifunctional protein deficiency in a blood spot from the newborn screening card by tandem mass spectrometry and DNA analysis. *Pediatr Res* 1999, 46:45-49.
7. Ushikubo et al. Molecular characterization of mitochondrial trifunctional protein deficiency: formation of the enzyme complex is important for stabilization of both [alpha] and [beta] subunits. *Am J Hum Genet* 1996, 58:979-988.
8. Ibdah et al. Mild trifunctional protein deficiency is associated with progressive neuropathy and myopathy and suggests a novel genotype-phenotype correlation. *J Clin Invest* 1998, 102(6):1193-9
9. Schulze et al. Expanded newborn screening for inborn errors of metabolism by electrospray ionization-tandem mass spectrometry: results, outcome, and implications. *Pediatrics* 2003, 111(6 Pt 1):1399-406.

Genes

[HADHA](#), [HADHB](#)

Indications

This test is indicated for:

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

Methodology

Next Generation Sequencing: In-solution hybridization of all coding exons is performed on the patient's genomic DNA. Although some deep intronic regions may also be analyzed, this assay is not meant to interrogate most promoter regions, deep intronic regions, or other regulatory elements, and does not detect single or multi-exon deletions or duplications. Direct sequencing of the captured regions is performed using next generation sequencing. The patient's gene sequences are then compared to a standard reference sequence. Potentially causative variants and areas of low coverage are Sanger-sequenced. Sequence variations are classified as pathogenic, likely pathogenic, benign, likely benign, or variants of unknown significance. Variants of unknown significance may require further studies of the patient and/or family members.

Detection

The vast majority of patients with clinical and biochemical diagnosis of MTP deficiency will have an abnormal DNA test.

Prevalence: TFP is rare with incidence estimates of 1:250,000 live births [9]. It is inherited in an autosomal recessive manner, therefore the recurrence risk for carrier parents of an affected child is 25%.

Next Generation Sequencing: Clinical Sensitivity: Unknown. Mutations in the promoter region, some mutations in the introns and other regulatory element mutations cannot be detected by this analysis. Large deletions/duplications will not be detected by this analysis. Results of molecular analysis should be interpreted in the context of the patient's clinical/biochemical phenotype. Analytical Sensitivity: ~99%.

Specimen Requirements

Submit only 1 of the following specimen types

Type: Saliva

Specimen Requirements:

Oragene™ Saliva Collection Kit

Oragene™ 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: 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: 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

Please 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. Contact the laboratory if further information is needed.

Related Tests

- [Urine Organic Acids \(OA\)](#) and [Plasma Acylcarnitine Profile \(AR\)](#) are used in the diagnosis of a patient with TFP deficiency.
- [Custom Diagnostics Known Mutation Analysis \(KM\)](#) is available to family members if mutations are identified by sequencing.
- For comprehensive testing, a [Trifunctional Protein Deficiency Deletion/Duplication Assay](#) is available separately. This test is indicated 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 to couples who are confirmed carriers of mutations. Please contact the laboratory genetic counselor to discuss appropriate testing prior to collecting a prenatal specimen.