

# A New Sporadic Point Mutation in the ATP6 Gene of the Mitochondrial DNA (mtDNA) in Leigh Disease

<sup>1</sup>Genevieve N. Langley, B.S., <sup>1</sup>Lauren C. Hyams, Ph.D., <sup>2</sup>Francis Dimario, M.D., <sup>1</sup>John M. Shoffner, M.D

<sup>1</sup>Medical Neurogenetics, LLC, Atlanta, GA; <sup>2</sup>Connecticut Children's Medical Center

## Background

Two mtDNA coded genes code essential polypeptides for Complex V (ATP synthase). Complex V is a multi-subunit enzyme of oxidative phosphorylation (OXPHOS) that synthesizes ATP by a rotary process that is driven by an electrochemical proton gradient across the inner mitochondrial membrane. The F<sub>0</sub> portion is responsible for delivering energy stored in the proton gradient to the F<sub>1</sub> portion for ATP synthesis. The F<sub>0</sub> contains mtDNA coded subunits: ATP6 and ATP8. At least six mutations in the ATP6 gene of the mtDNA can cause Leigh Disease. ATP6 mutations typically interfere with proton translocation by Complex V (ATP synthase) resulting in impaired ATP synthesis. In addition, reduced coupling of ATP synthesis by Complex V to proton translocation by Complexes I, III, and IV further impair oxidative phosphorylation function. Analysis of new mutations is important for understanding the relationship between genotype and phenotype.

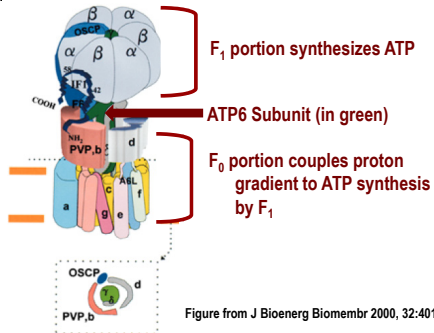


Figure from J Bioenerg Biomembr 2000, 32:401-411

## Clinical Description

- This patient was a 4 year 7 month old girl
- Patient had global developmental delay, proximal muscle weakness, tremor, intermittent dystonia, myoclonus.
- Brain MRI and spectroscopy were consistent with Leigh Disease.
- Growth: Head circumference about 40-50<sup>th</sup>%; Length 90-95<sup>th</sup>%; Weight ~10<sup>th</sup>%.
- Negative family history: Patient is a singleton case in the family, 2 healthy full siblings. 6 unaffected paternal half-siblings.

## Metabolic Studies

Plasma Lactate	20 (NL 3-12)
Blood Pyruvate	0.44 (NL 0.30-0.90)
	Increased Lactate
CSF Lactate and Pyruvate	Normal Pyruvate Normal Lactate/Pyruvate Ratio
CSF Amino Acids	Increased alanine

- Resting Metabolic Rate was decreased to 51% of normal. See poster P01.030 from our group for more discussion.
- The following labs were negative: Urine Amino Acids, Urine Organic Acids, Plasma Amino Acids, Blood Carnitine, Biotinidase, Phenylalanine loading test, Urine Glutamic Acid, muscle Coenzyme Q10 level, Pyruvate Dehydrogenase Complex enzymology.

Most patients with mitochondrial disease will have negative metabolic testing. In some patients the most significant abnormalities are observed in CSF.

## Muscle OXPHOS Enzymology

OXPHOS Enzyme Assay	Patient Activity (<5% level)	Mean±SD [5%-95%]
Complex I Assay (n-decyl CoQ electron acceptor)	0	85 ± 34 [47-160]
Complex I Assay (CoQ1 electron acceptor)	79	246 ± 118 [97-438]

OXPHOS enzymology was performed on fresh muscle. Defects were diagnosed by comparison of activity with the 5%-95% reference intervals calculated from 254 controls. Enzyme activity is expressed as nanomoles substrate/minute/mg of mitochondrial protein.

Due to complexities in disease pathogenesis, OXPHOS enzyme defects are highly variable even among groups of individuals who harbor identical mutations (1, 2). The activity measurements for OXPHOS enzymes depend in part on the stability of the individual OXPHOS enzymes, the functioning of individual enzyme subunits, as well as the presence of adequate supercomplex formation (aggregates of Complexes I, III, and IV). Complex V defects are often associated with Complex I defects.

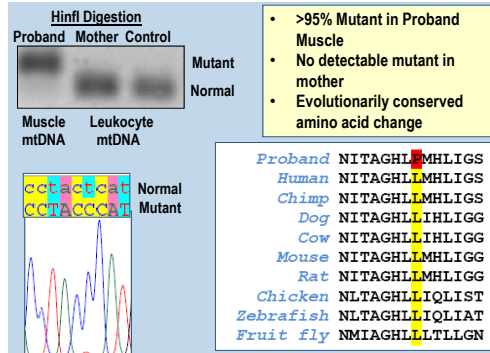
## OXPHOS Subunit Protein Chemistry

OXPHOS Enzyme Subunit Tested (Normalized to GAPDH)	Patient [<5% level]	Mean [5%-95%]
Complex I (ND6 subunit; mtDNA coded)	53.5%	71.8% [40.0%-103.6%]
Complex II (30 kDa subunit; nuclear DNA coded)	34.0%	40.0% [27.0%-53.0%]
Complex III (core 2 subunit; nuclear DNA coded)	58.5%	81.6% [48.2%-118.1%]
Complex IV (COX II subunit; mtDNA coded)	116.7%	136.3% [87.8%-184.7%]
Complex V (F1 alpha subunit; nuclear DNA coded)	84.8%	97.5% [64.7%-130.3%]

Patient OXPHOS subunit levels are compared to the control reference intervals (5%-95%) as well as to a concurrently assayed normal control and patient who harbors a pathogenic mtDNA mutation.

Patients with Complex V defects often have normal levels of OXPHOS subunits.

## ATP6 Mutation: 9035T>C Leucine (CTC) 170 Proline (CCC)



## Summary

- Leigh syndrome is an early-onset progressive neurodegenerative disorder with a characteristic neuropathology consisting of focal, bilateral lesions in one or more areas of the central nervous system, including the brainstem, thalamus, basal ganglia, cerebellum, and spinal cord. The lesions are areas of demyelination, gliosis, necrosis, spongiosis, or capillary proliferation. Clinical symptoms depend on which areas of the central nervous system are involved. The most common underlying cause for Leigh disease is an OXPHOS disease caused by nuclear DNA or mtDNA mutations.
- A new mutation causing Leigh disease was identified: T9035C Leucine (CTC) 170 Proline (CCC). The mutation appeared essentially homoplasmic in muscle mtDNA from the proband. Leukocyte mtDNA from the mother harbored only mtDNA with a normal sequence. A sporadic event or even germ cell line mosaicism are possibilities. A sporadic event is likely given that the two siblings are unaffected. Since disease manifestations can occur at later ages in patients with lower levels of mutant mtDNA, siblings require monitoring over time. Since the siblings are asymptomatic and <18 years of age, they have not been tested.
- The sporadic occurrence emphasizes the importance of careful investigation of singleton cases for mtDNA mutations.
- Due to the complex nature of oxidative phosphorylation biochemistry (eg. supercomplex formation, monomeric enzyme assembly) and genetics (eg. mtDNA mutations, heteroplasmy, homoplasmy), OXPHOS testing may NOT predict the precise location of the mutation. Multi-faceted approach are excellent at identifying OXPHOS defects.

## References

- Shoffner JM, Lott MT, Lezza AM, Seibel P, Ballinger SW, Wallace DC. Myoclonic epilepsy and ragged-red fiber disease (MERRF) is associated with a mitochondrial DNA (rRNA(Lys) mutation. Cell 1990;61:931-937.
- Wallace DC, Zheng XX, Lott MT, Shoffner, J.M., et al. Familial mitochondrial encephalomyopathy (MERRF): genetic, pathophysiological, and biochemical characterization of a mitochondrial DNA disease. Cell 1988;55:601-610.