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A Novel Heteroplasmic Cytochrome b Mutation in the Mitochondrial DNA is Associated with MELAS (Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-Like Episodes)[P03.051]

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Background

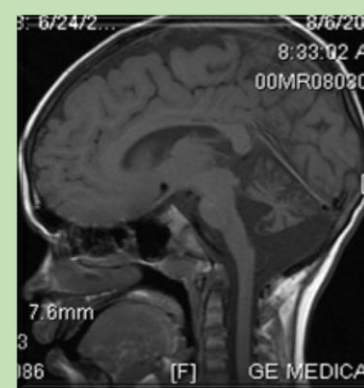
Oxidative phosphorylation (OXPHOS) is critical to cellular function as the primary source for energy (ATP) in most cell types, the control point for cellular redox, and as a control point for essential metabolic and signaling pathways that range from the synthesis of pyrimidines to the regulation of apoptosis.

Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) is a mitochondrial disease that is most commonly caused by a point mutation in the mitochondrial DNA (mtDNA) within a transfer RNA gene. We describe a patient with clinical features consistent with MELAS who harbors a mtDNA point mutation in the cytochrome b subunit of Complex III.

Point mutations in the mtDNA coded cytochrome b gene are typically somatic cell lineage specific, are sporadic, and are associated with progressive myopathy (with or without rhabdomyolysis). Small deletions in this gene can have more diverse phenotypes including parkinsonism/MELAS overlap syndrome and myopathies (see Poster P05.130). (1-2) A patient with MELAS is characterized who harbors a heteroplasmic point mutation in the cytochrome b gene.

Clinical

Delayed development (Fine and gross motor)
Progressive cognitive decline
Migraine headaches
Significant fatigue with activity
Stroke-like episodes beginning at 4 ½ years of age
Progressive cerebellar atrophy



Family history: Negative

Metabolic abnormalities:

Reduced Resting Metabolic Rate: 66% of Predicted
Increased urine lactate
Increased plasma lactate and pyruvate
Increased lactate/pyruvate ratio in CSF
Increased CSF alanine

Unremarkable Studies:

Pyruvate dehydrogenase complex (PDHC) enzymology
Normal PDHC Western blot
Muscle Histology

Cytochrome b Point Mutation: 15635 T>C, Serine 297 Proline

Proband: Heteroplasmic in muscle; Mother: ABSENT in Leukocyte mtDNA

Frequency in a multi-ethnic sample population (~3000 individuals) is 0.03% which is comparable to the frequencies of various pathogenic mtDNA mutations.

Highly evolutionarily conserved amino acid

Human	LALLSILILAMIP
Pan troglodytes	LALLSILILTAIP
Canis lupus familiaris	LALVFSILILAFIP
Bos taurus	LALAFSILILALIP
Mus musculus	LALILSILILALMP
Rattus norvegicus	VALILSILILAFIP
Gallus gallus	LALAASVLILFLIP
Danio rerio	LALLFSILVLMVVP
Drosophila melanogaster	IALVLSIAILMLIP
Anopheles gambiae	IALVLSIAILLILP
Caenorhabditis elegans	IALLMSIVTFYFFA
Schizosaccharomyces pombe	IAMLLSILVLLLLP

Biochemistry

Fibroblast High Resolution Respirometry

	Range	5%	25%	Mean	75%	95%	Proband
Uncoupling Ratio	2.30-3.13	2.28	2.52	2.70	2.87	3.12	1.98
Net Routine Flux Control Ratio	0.32-0.44	0.32	0.35	0.37	0.40	0.43	0.51
Respiratory Control Ratio	7.60-14.88	7.10	9.28	10.79	12.30	14.5	8.21
Leak Flux Control Ratio	0.07-0.13	0.06	0.08	0.10	0.11	0.13	0.12
Phosphorylation Respiratory Control Ratio	0.22-0.36	0.22	0.25	0.28	0.30	0.34	0.38

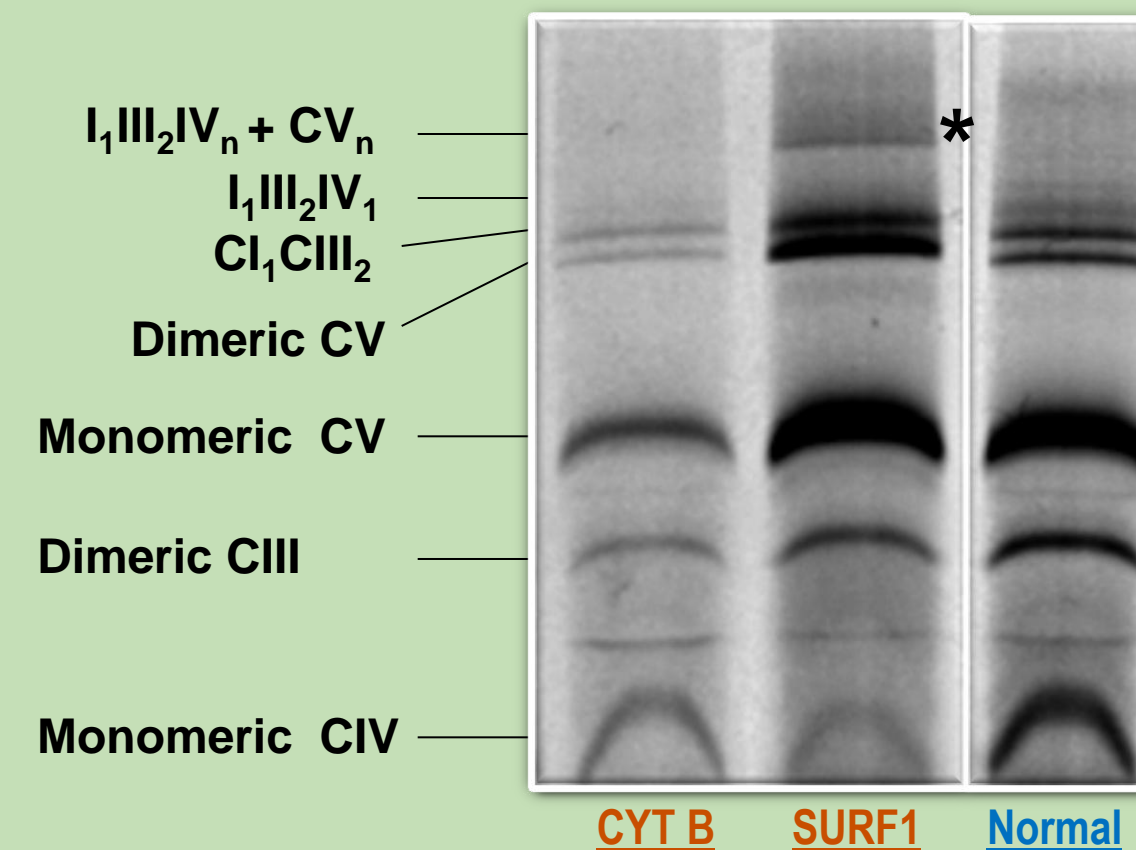
Abnormal values are shaded. Abnormal Uncoupling ratio and Net Routine Flux Control Ratio indicating a diminished capacity of the cells to respond to increased ATP demands (i.e. decreased reserve capacity). Increased Phosphorylation potential indicating a decline of cellular respiration without a significant effect on the rate of oxidative phosphorylation.

OXPHOS Enzymology

Complex I and Complex III were decreased below the 5% reference intervals.

Complex III defects commonly produce combined abnormalities in Complex I and Complex III due to impaired supercomplex formation.

Blue Native Gel Analysis

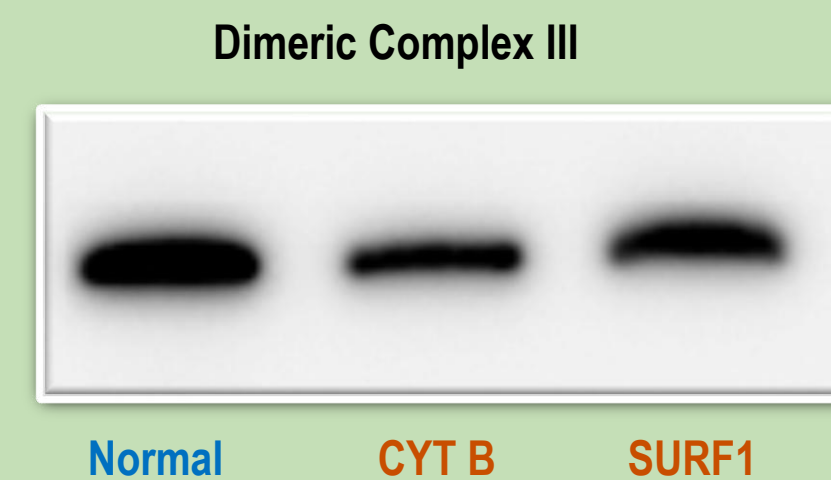


Blue Native Gel Electrophoresis

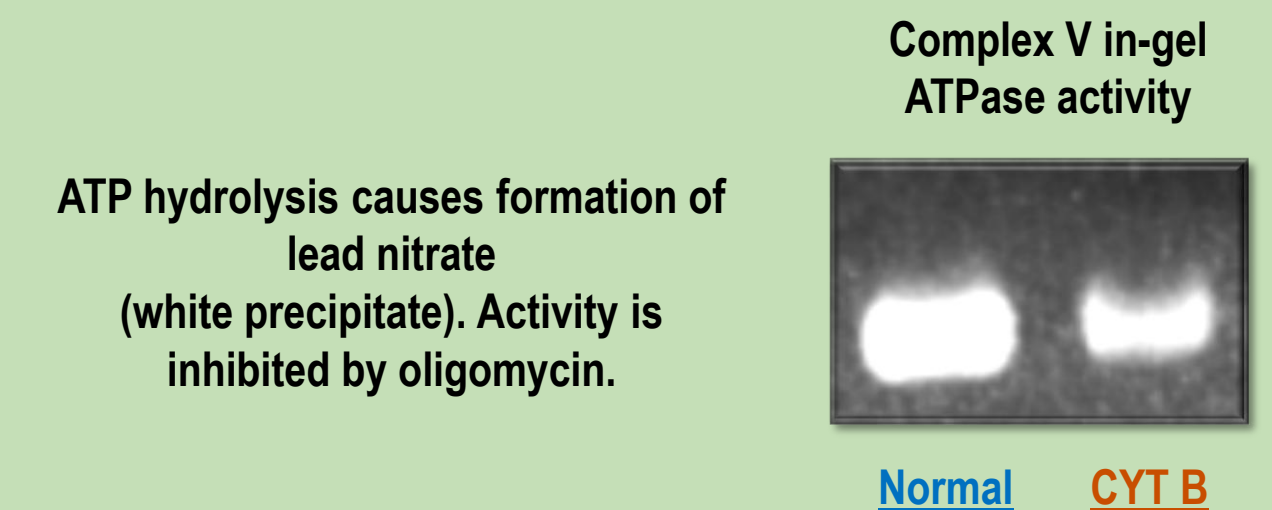
- Decreased supercomplex formation is identified in the MELAS patient (CYT B) and in an abnormal control (adult with SURF1 mutation). (CI₁CIII₂; I₁III₂IV₁; I₁III₂IV_n) The higher molecular weight bands (asterisk) in the SURF1 patient may be oligomeric forms of Complex V. Note that in the Adult patient with SURF1 mutations, monomeric Complex IV is only mildly decreased.
- Dimeric Complex III and monomeric Complex V appears intact but may be decreased relative to control tissue.

Clear Native Gel Immunoblot

Assembly of Complex I, Complex II, Complex III, Complex IV and Complex V was appropriate. An example of a Clear Native Gel Immunoblot is given below. Dimeric Complex III is shown for the proband, a normal control, and for an Adult patient harboring a mutation in the SURF1 gene.



Clear Native In-Gel Enzymology



Clear Native In-Gel ATPase Activity

- A decrease in ATPase activity may be present.

Summary

- A singleton case with a MELAS phenotype harbored a heteroplasmic point mutation in the mtDNA within the cytochrome b subunit of Complex III.
- The mutation was present in proband muscle but was not present in the leukocyte mtDNA from the mother. The mutation appears to be sporadic. Family history was unremarkable.
- OXPHOS supercomplex analysis and monomeric enzyme analysis showed the following features.
 - Decreased supercomplex formation (CI₁CIII₂; I₁III₂IV₁; I₁III₂IV_n)
 - Possible decrease of oligomeric forms of Complex V.
 - Although monomeric enzyme assembly of Complexes I-V appeared to be intact, the complexes appeared decreased relative to controls, particularly for Complexes III and V.
- OXPHOS enzymology was consistent with the supercomplex data. As supercomplex formation decreases, decreases in enzyme activity measurements of Complex I and Complex III become detectable. This data is consistent with the abnormalities observed with high resolution respirometry.
- The ATPase activity of Complex V appeared decreased suggesting that Complex V may be dysfunctional. The mechanism is unclear.

Selected References

- De Coo IF, Renier WO, Ruitenbeek W, Ter Laak HJ, Bakker M, Schagger H, Van Oost BA, Smeets HJ. A 4-base pair deletion in the mitochondrial cytochrome b gene associated with parkinsonism/MELAS overlap syndrome. Ann Neurol. 1999 Jan;45(1):130-3.
- Andreu AL, Hanna MG, Reichmann H, Bruno C, Penn AS, Tanji K, Pallotti F, Iwata S, Bonilla E, Lach B, Morgan-Hughes J, DiMauro S. Exercise intolerance due to mutations in the cytochrome b gene of mitochondrial DNA. N Engl J Med. 1999 Sep 30;341(14):1037-44.