

Effects of a Novel Complex III (cytochrome b subunit) Mutation on Supercomplex Formation in a Patient with Mitochondrial Myopathy

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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. Optimal OXPHOS function requires aggregation of individual OXPHOS enzymes into supercomplexes which allows efficient and rapid transport of electrons. Supercomplexes allow efficient formation of an electrochemical (proton) gradient created by Complexes I, III, and IV that is then used by Complex V to synthesize ATP. In many classes of mitochondrial disease, impairment of the monomeric enzymes (Complexes I-V) and supercomplex assembly occurs. Functional supercomplexes contain a single Complex I enzyme, two Complex III enzymes, and variable numbers of Complex IV enzymes (Complexes I+III₂+IV) plus the mobile electron carriers CoQ10 and cytochrome c. Complex II also can be associated with the Complex I+III₂+IV structure. During isolation of supercomplexes, other classes of supercomplexes are observed: (1) Complexes I+III₂+V; (2) Complexes I+III₂+IV; (3) Complexes III+IV. The role of these other supercomplex classes, particularly those lacking Complex IV are unknown, but they may be intermediate structures involved in functional supercomplex assembly.

The effects of gene mutations involving Complex III subunits on supercomplex formation has been investigated in only a few cases. (1-3) To date all patients show impaired assembly of supercomplexes as well as assembly of Complex I. Complex III is required for appropriate assembly of Complex I. A novel 9 base pair (3 amino acid) in-frame deletion in the cytochrome b subunit of Complex III is identified and the effects on monomeric enzyme assembly and supercomplex formation are investigated.

Clinical Description

9 year old boy:

- Severe fatigue with activity
- Myalgias
- Rare muscle cramps
- Muscle weakness
- Short Stature

- Normal cardiac evaluation
- Increased Resting Metabolic Rate: 128% of normal.
- Improvement with Leucovorin and CoQ10 treatment

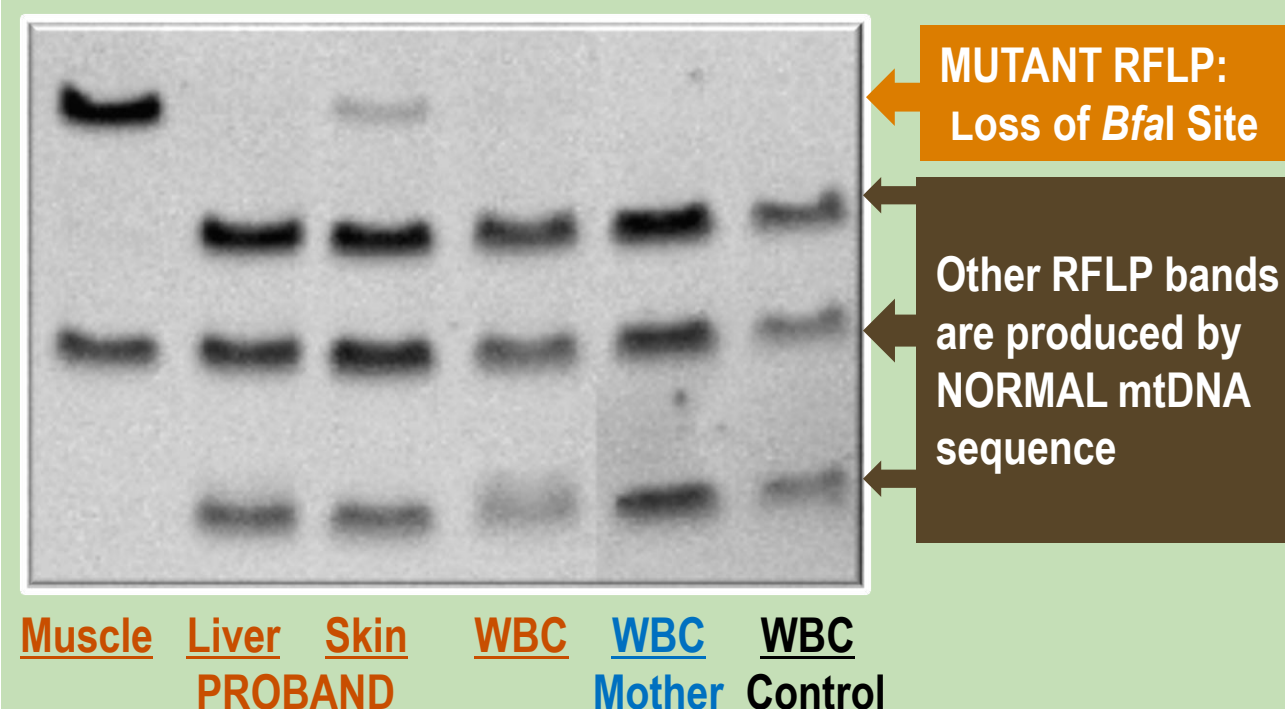
Laboratory:

- HyperCKemia (normal to 1400 Units/Liter)
- Increased Lactate, Pyruvate (Blood, Urine, CSF)

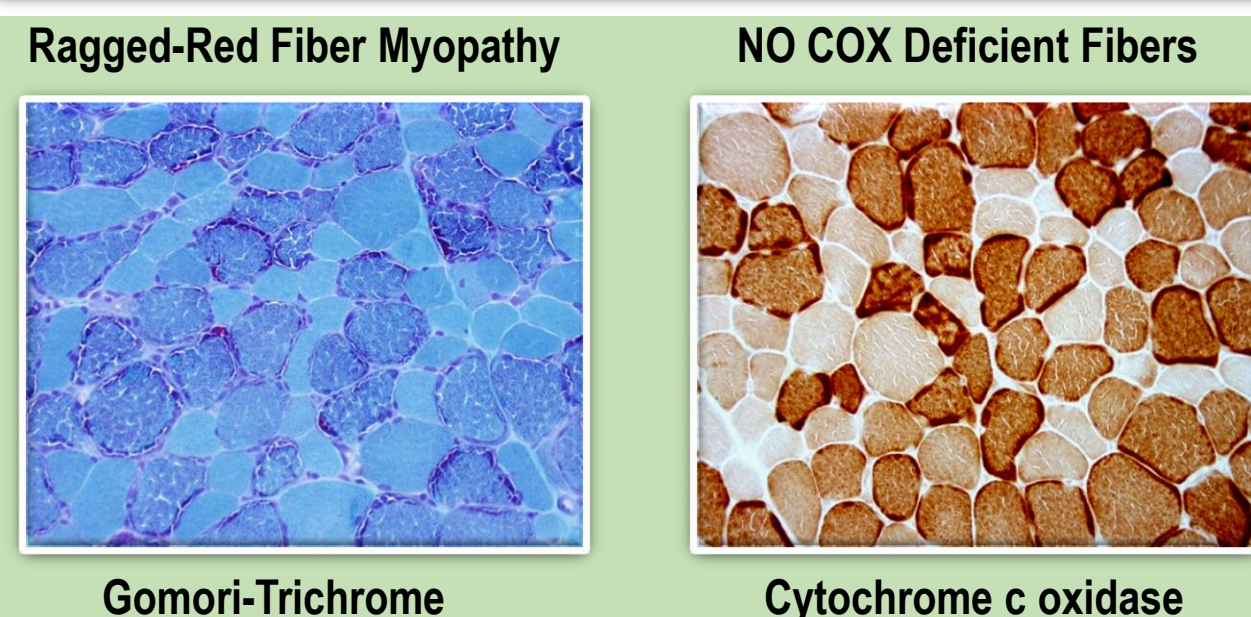
- Increased Alanine (Blood, CSF)
- Cerebral Folate Deficiency

Cytochrome b Mutation: 15319_15327delCCTAGCAAC

We identified a novel 9 base pair in-frame deletion in the cytochrome b gene of the mtDNA by direct sequencing: 15319_15327delCCTAGCAAC. The mutation is identified in muscle and skin. Restriction fragment length polymorphism (RFLP) testing of proband muscle DNA and the mother's leukocyte DNA showed that the mutation is only present in the proband and was essentially homoplasmic in the proband's muscle, heteroplasmic in skin, and undetectable in liver and leukocytes (WBC).



Muscle Histology/Enzymology



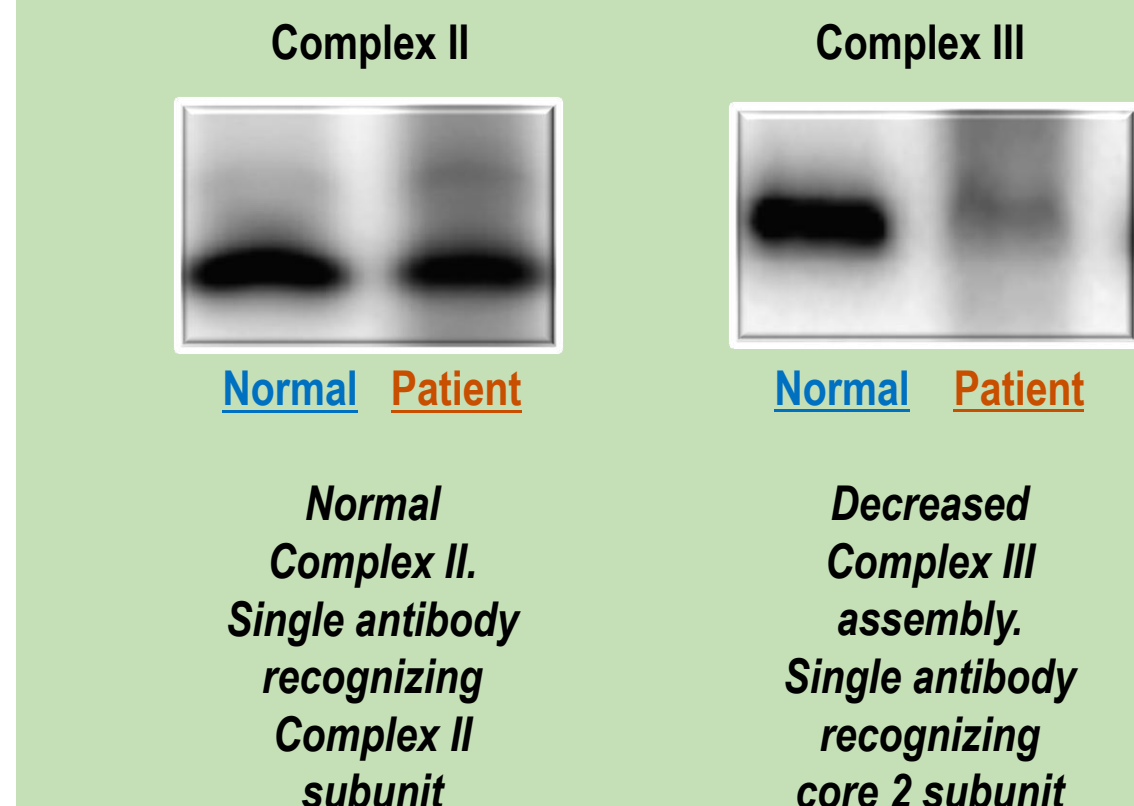
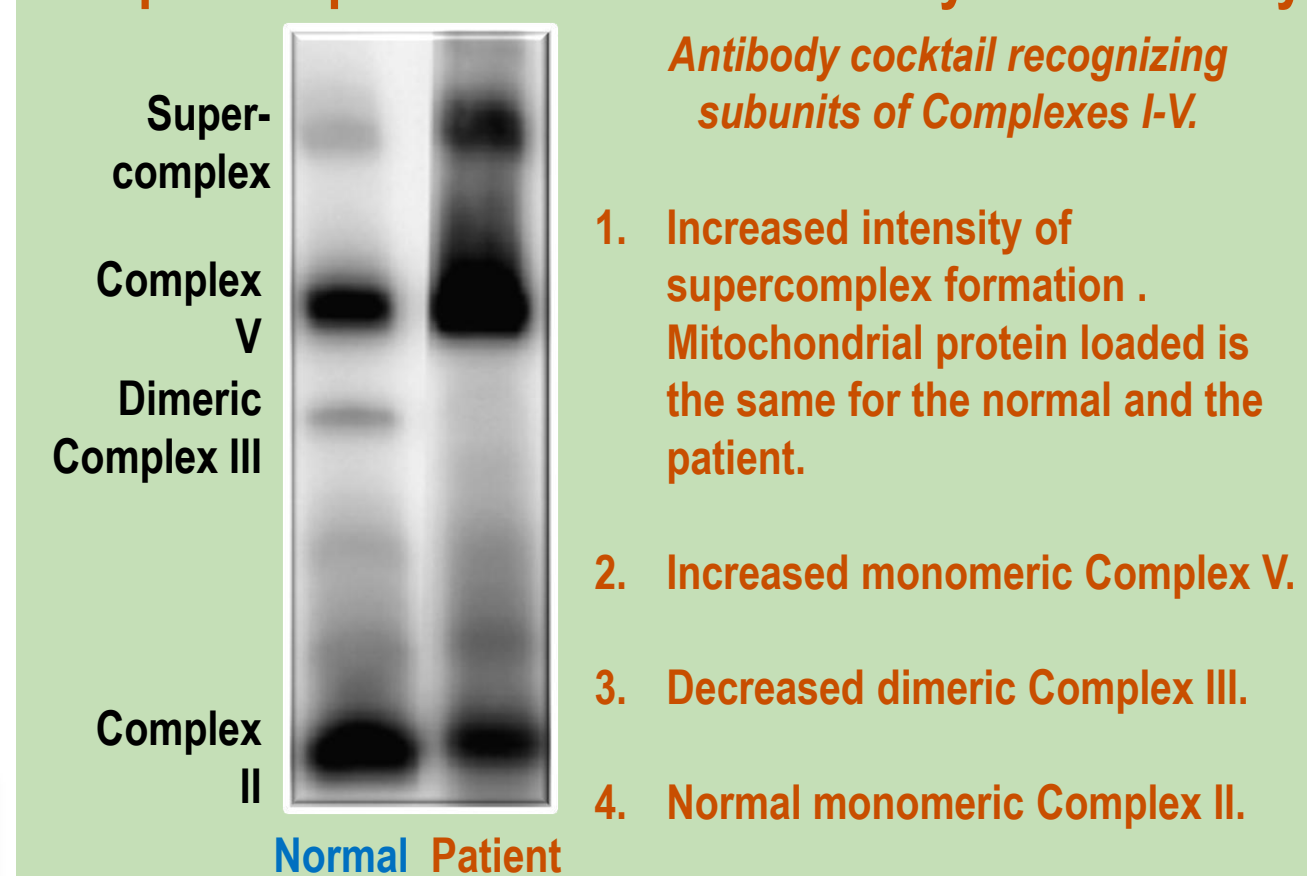
OXPHOS Enzymology revealed an ISOLATED Complex III Defect:
455 nanomoles substrate/minute/mg mitochondrial protein
[Normal 5%-95% Reference Interval: 837 – 2368nmol/min/mg]

OXPHOS Supercomplex Formation and Monomeric Enzyme Assembly

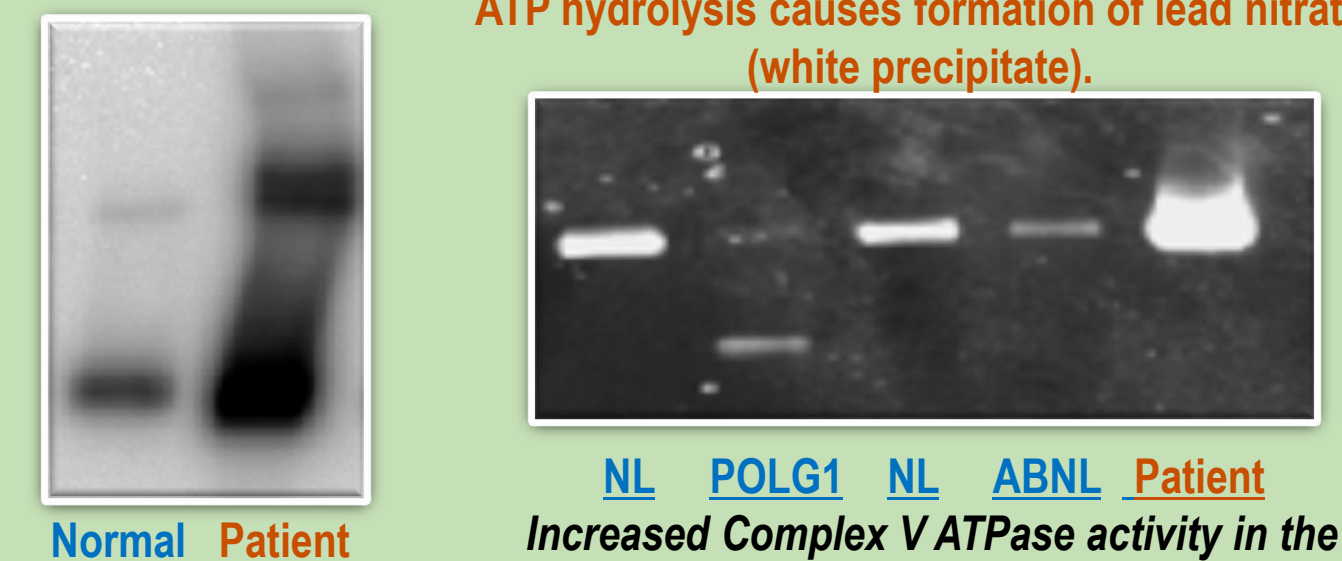
OXPHOS supercomplex formation and monomeric enzyme assembly was investigated in MUSCLE mitochondria by Blue Native Gel analysis, by Clear Native Immunoblot analysis, and by Clear Native Gel Enzymology.

Blue Native Gel Analysis demonstrated an increase in supercomplex formation and a decrease in Complex III assembly. Clear Native Immunoblot was used to investigate these findings further.

Supercomplexes + Monomeric Enzyme Assembly



Complex V: In-Gel ATPase Activity



Increased Complex V (Monomers and Oligomers)

Increased Complex V ATPase activity in the patient due to increased Complex V expression. Note comparison with normals (NL) and patient harboring a POLG1 mutation. POLG1 shows dissociation of the F₁ from F₀ portions of Complex V. Activity is inhibited by oligomycin.

Summary

1. A novel in-frame cytochrome b gene mutation is described that removes three amino acids. This mutation produces a mitochondrial disease characterized primarily by neuromuscular symptoms and an enzymologic defect in Complex III activity. Although the CSF lactate, pyruvate, and alanine is elevated, cognitive symptoms have not been recognized but may develop over time. Significant variation in the concentration of the mutation among tissues was present.
2. Significant numbers of ragged-red fibers were present in the patient's muscle suggesting that cytochrome b dysfunction could be critical to the genesis of this unique histologic change.
3. OXPHOS supercomplex analysis and monomeric enzyme analysis showed the following features:
 - a. Increased supercomplex formation which may indicate that the mutation enhances OXPHOS enzyme aggregation.
 - b. Decreased amounts of dimeric Complex III. The Complex III formed could be primarily bound in supercomplexes which is suggested by Clear Native gel assessment using only antibody to a Complex III subunit.
 - c. Increased Complex V oligomers and monomers along with increased ATPase activity. This feature could account for the increased resting metabolic rate.

Selected References

1. Acin-Perez, R., M. P. Bayona-Bafaluy, et al. (2004). "Respiratory complex III is required to maintain complex I in mammalian mitochondria." Mol Cell 13(6): 805-15.
2. Acin-Perez, R., P. Fernandez-Silva, et al. (2008). "Respiratory active mitochondrial supercomplexes." Mol Cell 32(4): 529-39.
3. D'Aurelio, M., C. D. Gajewski, et al. (2006). "Respiratory chain supercomplexes set the threshold for respiration defects in human mtDNA mutant cybrids." Hum Mol Genet 15(13): 2157-69