

SURF1 mutation: Effects on Supercomplex Formation and Monomeric Enzyme Assembly [P05.128]

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Lauren Mylacraine, B.S., Genevieve N. Langley, B.S., Lauren C. Hyams, Ph.D.,
John M. Shoffner, M.D. Medical Neurogenetics, LLC, Atlanta, GA

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 structure involved in functional supercomplex assembly.

The effects of gene mutations involving Complex IV (cytochrome c oxidase) on supercomplex formation has been investigated in only a few cases harboring SURF1, COX10, and SCO1 mutations. (1-3) To date all patients with mutations in these genes show impaired assembly of supercomplexes. Assessment of patients with SURF1 mutations demonstrates diverse effects on OXPHOS function.

Clinical

Three patients with Leigh disease and who harbor SURF1 mutations:

Patient 1: Pediatric Leigh Disease

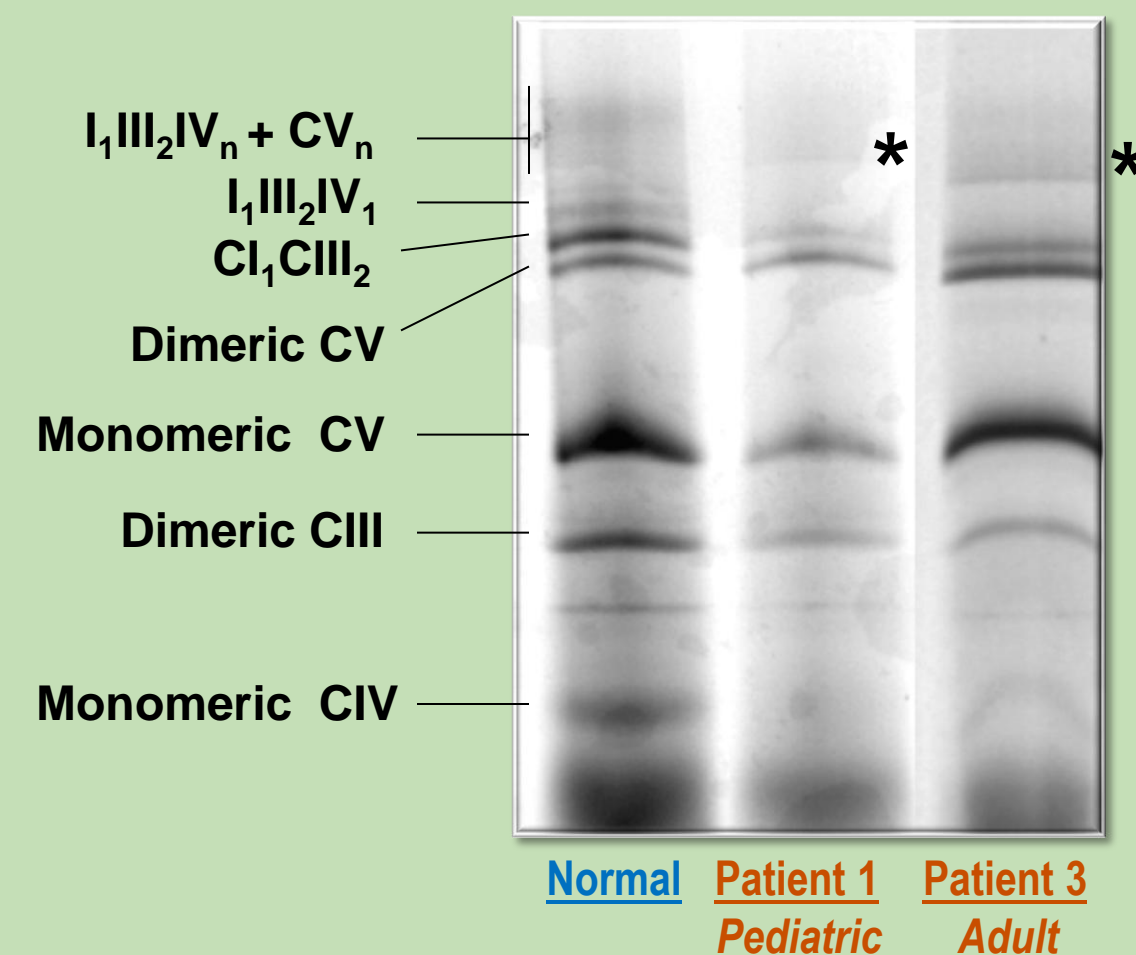
Patient 2: Pediatric Leigh Disease

Patient 3: Adult Leigh Disease

Blue Native Gel Analysis

OXPHOS supercomplex formation and monomeric enzyme assembly was investigated in MUSCLE mitochondria by Blue Native Gel electrophoresis. Clear Native Gel electrophoresis provides higher resolution of Complex IV (cytochrome c oxidase) assembly.

Supercomplexes + Monomeric OXPHOS Enzyme Assembly



Blue Native Gel Electrophoresis (2 different gels)

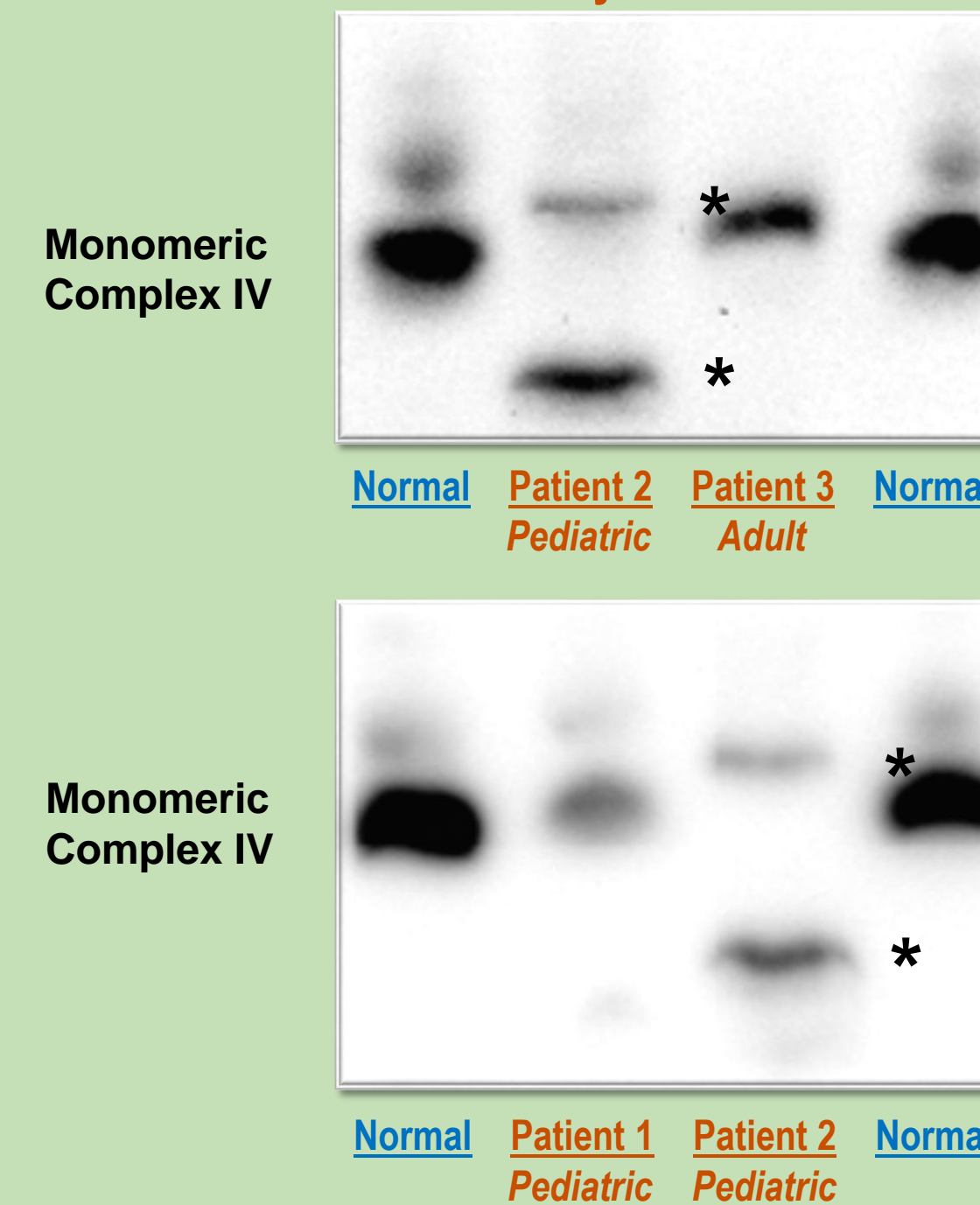
CI = Complex I; CIII = Complex III; CIV = Complex IV; CV = Complex V

1. Decreased supercomplex formation is identified in Patients 1 and 3 (CI₁CIII₂; I₁III₂IV₁; I₁III₂IV_n)
2. Decreased assembly of Complex IV is present in Patient 1 and Patient 3. The adult (Patient 3) has more Complex IV assembly than the pediatric patient (Patient 1).
3. Pediatric Patient 1: Monomeric Complex V, and dimeric Complex III formation appear decreased. Monomeric Complex II formation appeared unaffected.
4. Pediatric Patient 2: Insufficient muscle for analysis.
5. Adult Patient 3: Dimeric Complex V, dimeric Complex III, and monomeric Complex V appear unaffected.
6. The higher molecular weight bands (asterisk) may be oligomeric forms of Complex V.

Clear Native Gel Immunoblot

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

Complex IV Monomeric OXPHOS Enzyme Assembly

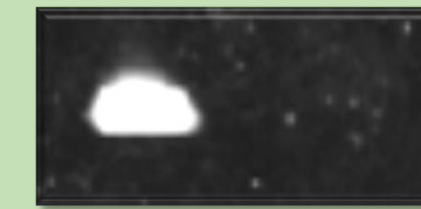


Clear Native Gel Electrophoresis (2 different gels)

1. Decreased assembly of monomeric Complex IV
2. Pediatric Patient 1: Decreased monomeric Complex IV assembly.
3. Pediatric Patient 2: Decreased monomeric Complex IV assembly with abnormal higher and lower molecular weight forms of Complex IV (asterisks).
4. Adult Patient 3: Complex IV assembly may be abnormal (migration at slightly higher molecular weight). Assembly of monomeric Complexes I, II, III, and V appeared intact (data not shown)

Clear Native In-Gel Enzymology

Complex V in-gel ATPase activity
ATP hydrolysis causes formation of lead nitrate (white precipitate)



Decreased ATPase Activity.

Normal Patient 1
Pediatric

Clear Native In-Gel ATPase Activity

1. Pediatric Patient 1: Decreased ATPase activity
2. Pediatric Patient 2: Insufficient muscle for analysis
3. Adult Patient 3: Intact ATPase activity

Summary

1. Autosomal recessive mutations in the SURF1 gene are a common cause of Leigh disease. SURF1 is an assembly factor. Complex IV function is severely abnormal due to impaired Complex IV assembly.
2. Mutations in Complex IV assembly factors such as SURF1, COX10 and SCO1 are easily recognized by the diffuse decrease in Complex IV activity observed by histochemical, immunofluorescence, enzymology, and protein chemistry approaches.
3. OXPHOS supercomplex analysis and monomeric enzyme analysis showed the following features:
 - a. Decreased supercomplex formation (CI₁CIII₂; I₁III₂IV₁; I₁III₂IV_n)
 - b. Monomeric Complex IV is highly abnormal showing decreased assembly as well as abnormal high and low molecular weight Complex IV structures. These abnormal Complex IV structures likely represent abnormally assembled and dysfunctional Complex IV.
 - c. In severe cases, Complex V appears to be affected. When the whole Complex V (ATP synthase) enzyme is isolated for Clear Native In-Gel enzymological analysis, patients can show impaired ATPase activity. This finding suggests that Complex V may be secondarily affected in some SURF1 patients thus contributing to phenotypic variation observed among these patients.

Selected References

1. Williams, S. L., I. Valnot, et al. (2004). "Cytochrome c oxidase subassemblies in fibroblast cultures from patients carrying mutations in COX10, SCO1, or SURF1." *J Biol Chem* 279(9): 7462-9
2. Diaz, F., H. Fukui, et al. (2006). "Cytochrome c oxidase is required for the assembly/stability of respiratory complex I in mouse fibroblasts." *Mol Cell Biol* 26(13): 4872-81
3. Acin-Perez, R., P. Fernandez-Silva, et al. (2008). "Respiratory active mitochondrial supercomplexes." *Mol Cell* 32(4): 529-39