

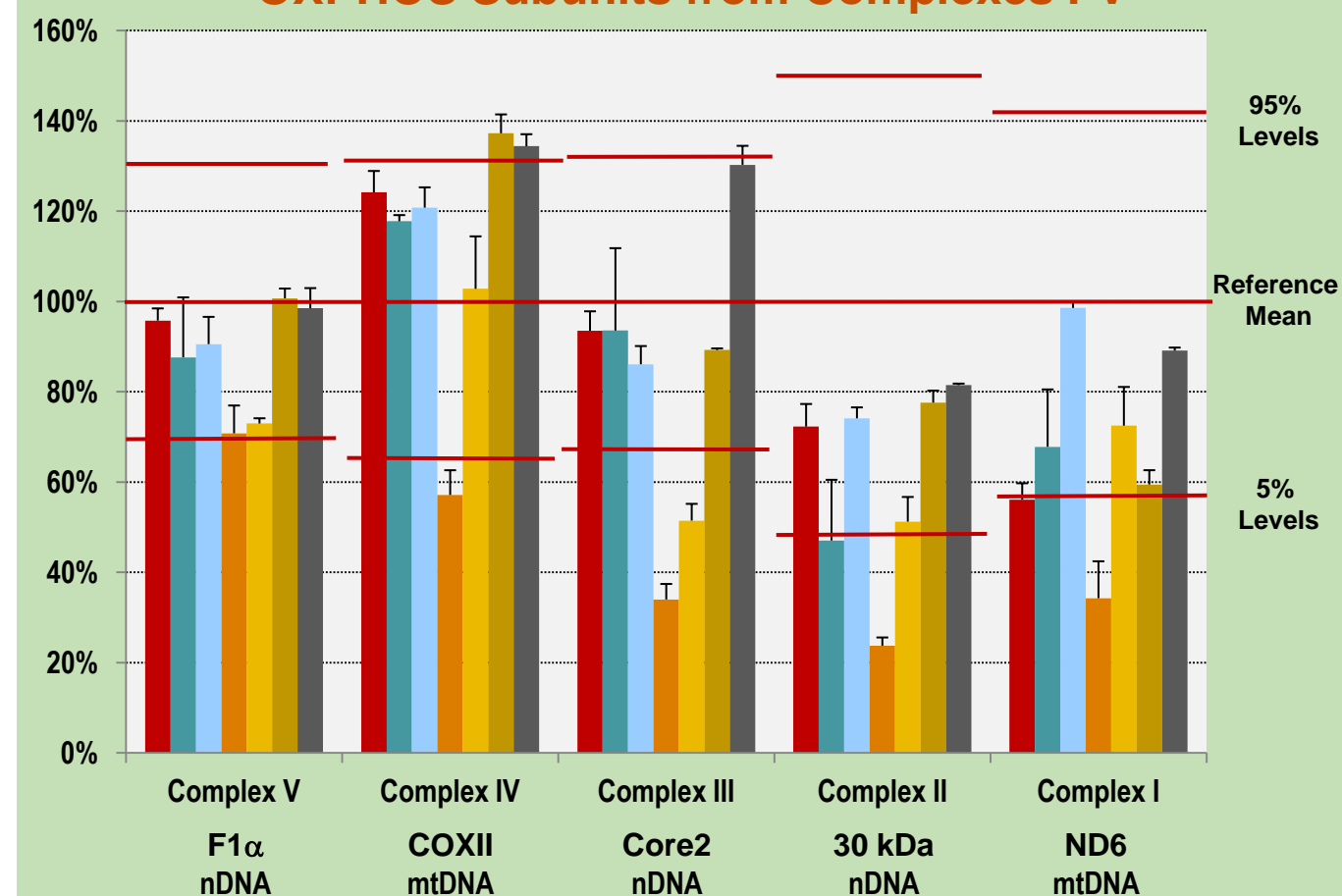
## Background

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.

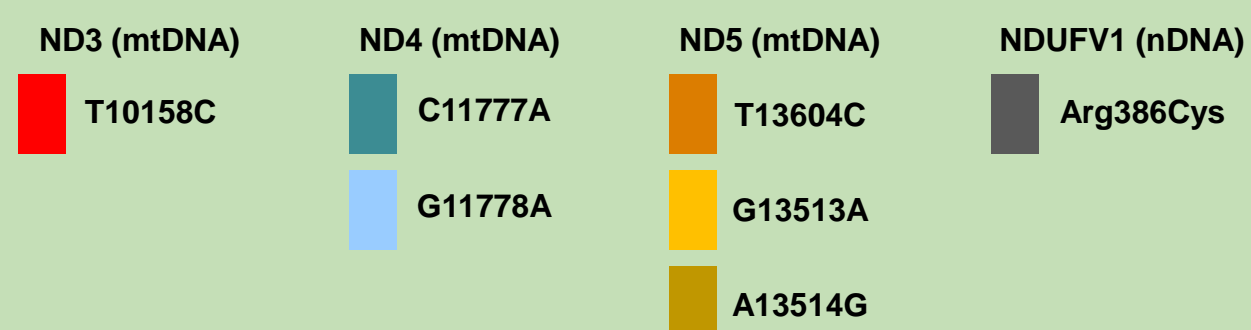
The activity measurements obtained from OXPHOS enzymology depends in part on the stability of the individual OXPHOS enzymes, the functioning of individual enzyme subunits, as well as the presence of adequate supercomplex formation (aggregates of Complexes I, III, and IV). Heterogeneous categories of OXPHOS enzyme defects are commonly observed in patients with known mtDNA or nuclear DNA mutations in genes important for proper OXPHOS function. Due to complexities in disease pathogenesis, OXPHOS enzyme defects are highly variable even among groups of individuals who harbor identical mutations (1-2).

## Complex I Mutations

### MUSCLE : Western Blot Assessment of Selected OXPHOS Subunits from Complexes I-V

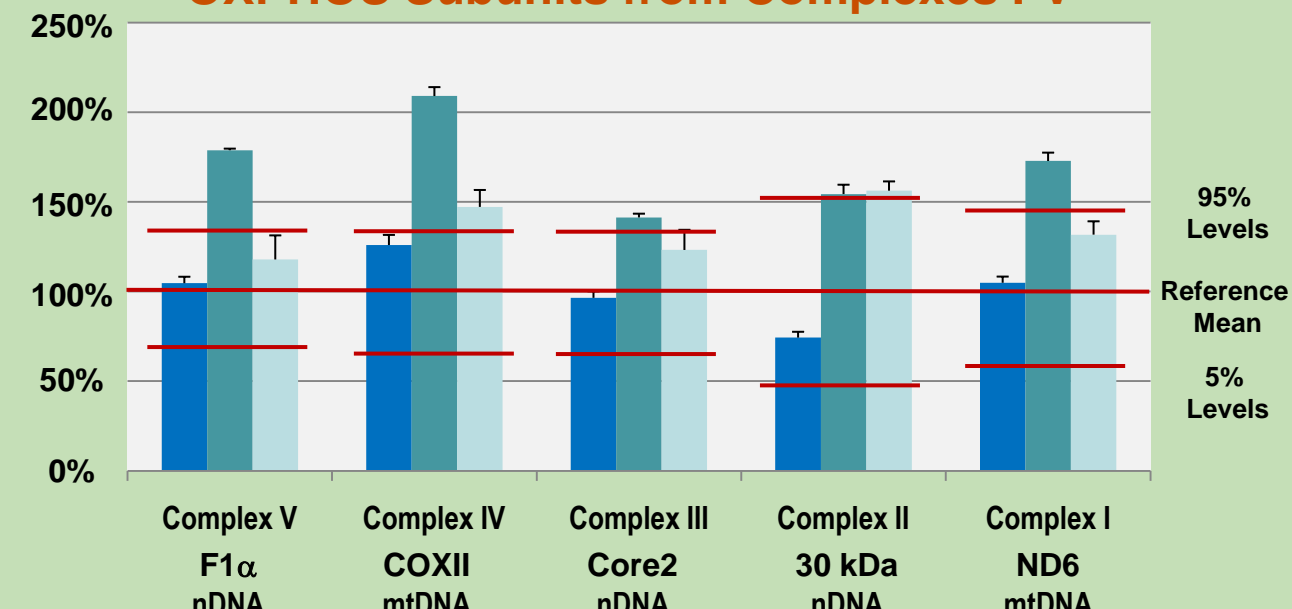


Error bars = SEM; Triplicate determinations  
Data for each Complex I-V is expressed as percentage of the reference interval mean. 5% and 95% reference intervals are shown (upper and lower red lines).



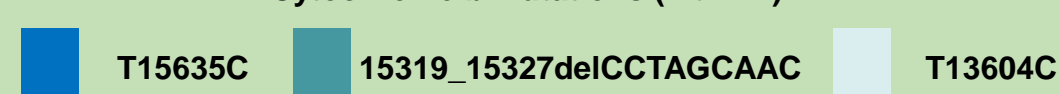
## Complex III Mutations

### MUSCLE : Western Blot Assessment of Selected OXPHOS Subunits from Complexes I-V



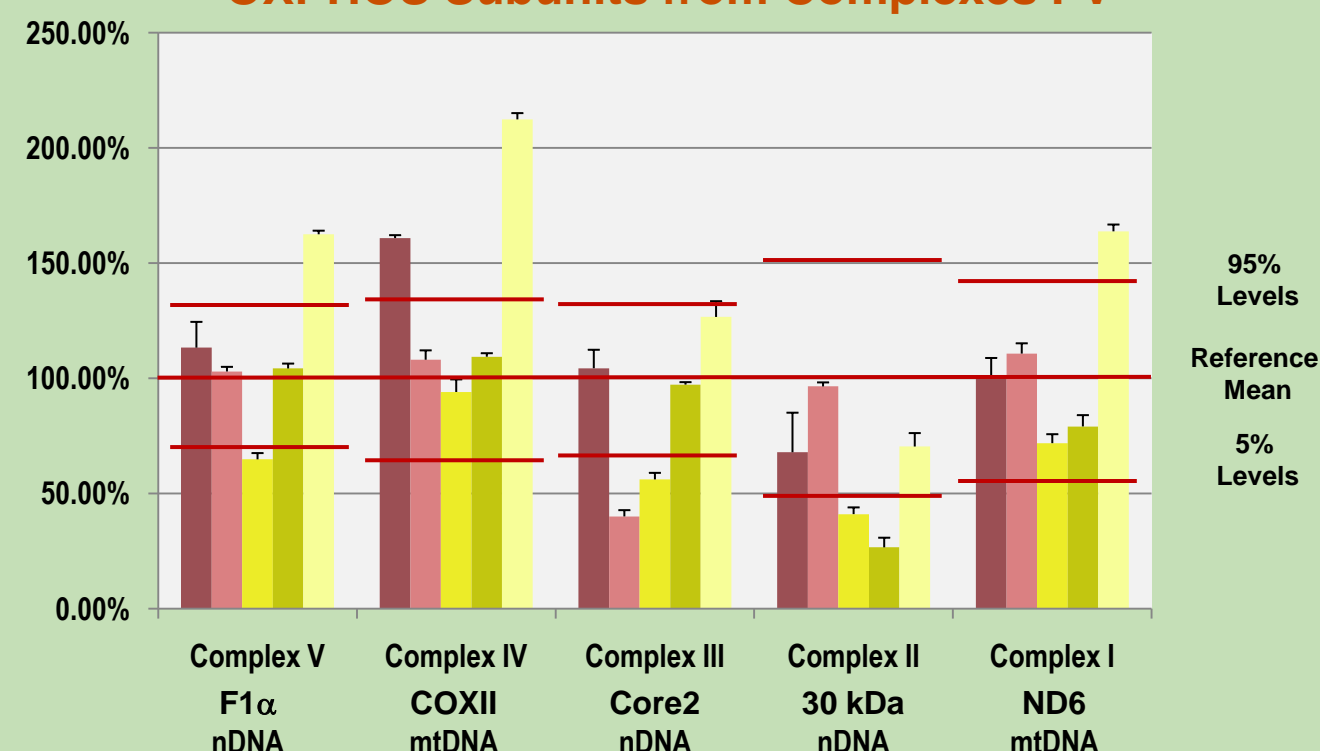
Error bars = SEM; Triplicate determinations  
Data for each Complex I-V is expressed as percentage of the reference interval mean. 5% and 95% reference intervals are shown (upper and lower red lines).

#### Cytochrome b Mutations (mtDNA)

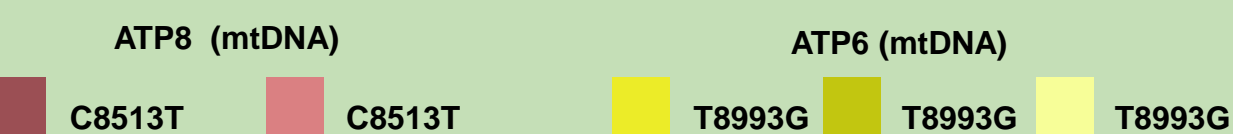


## Complex V Mutations

### MUSCLE : Western Blot Assessment of Selected OXPHOS Subunits from Complexes I-V

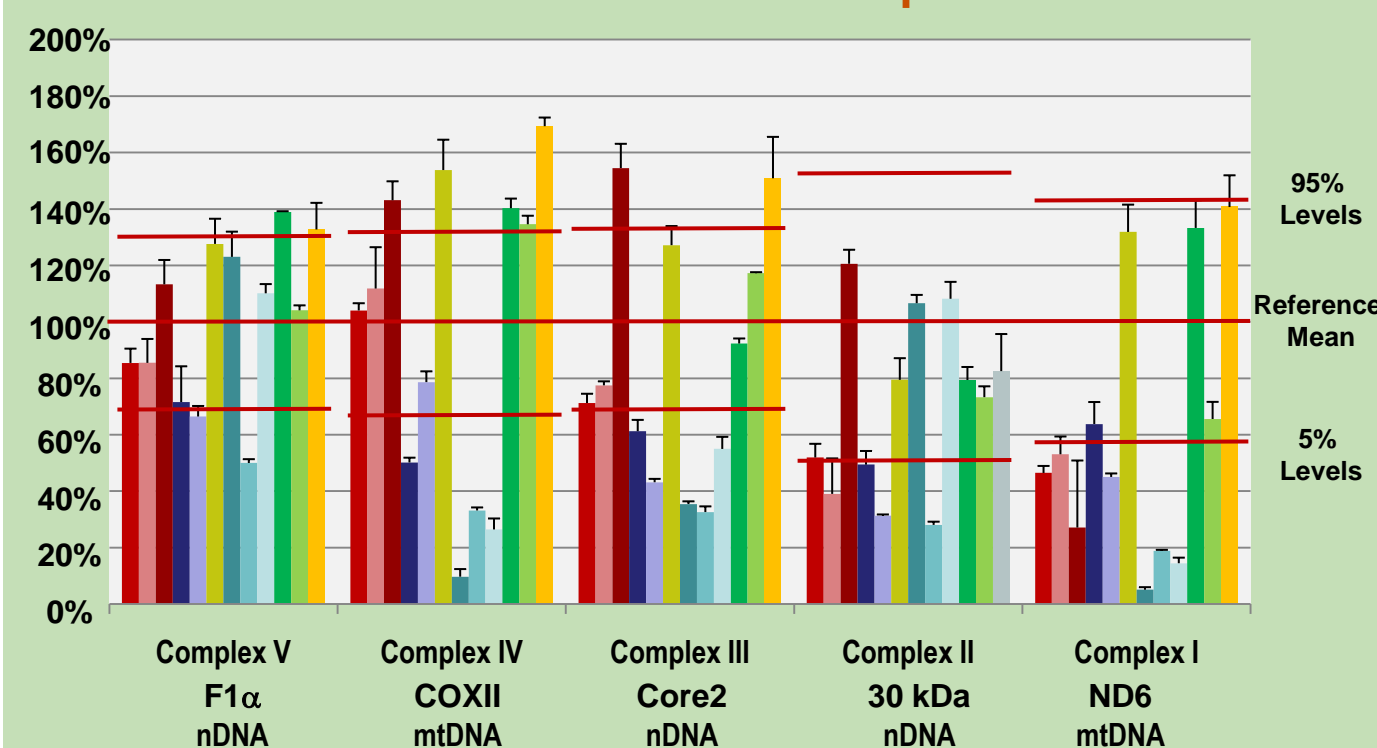


Error bars = SEM; Triplicate determinations  
Data for each Complex I-V is expressed as percentage of the reference interval mean. 5% and 95% reference intervals are shown (upper and lower red lines).

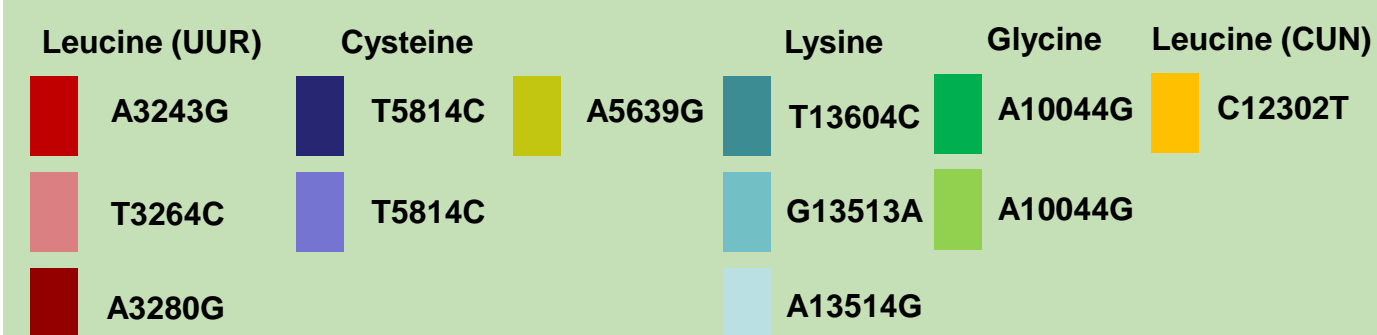


## Transfer RNA Mutations

### MUSCLE : Western Blot Assessment of Selected OXPHOS Subunits from Complexes I-V

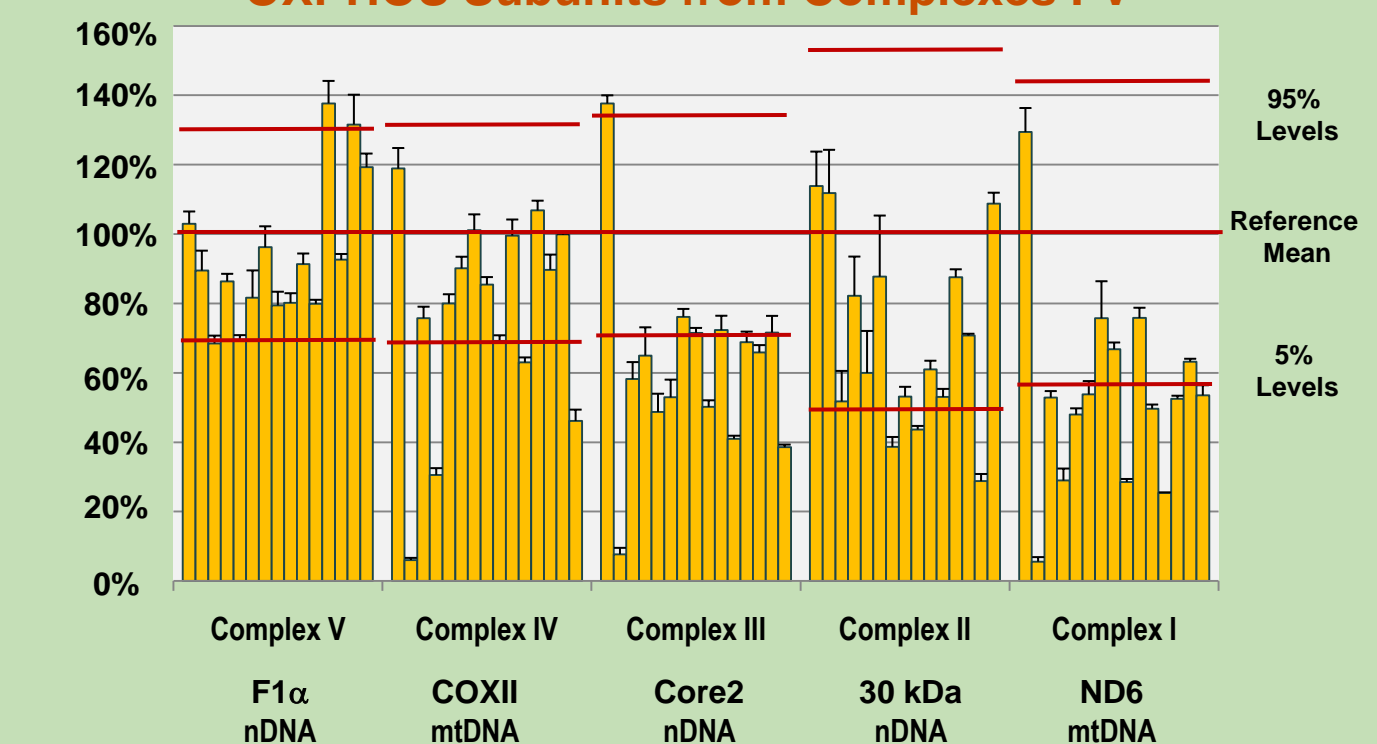


Error bars = SEM; Triplicate determinations  
Data for each Complex I-V is expressed as percentage of the reference interval mean. 5% and 95% reference intervals are shown (upper and lower red lines).



## mtDNA Deletion Mutations

### MUSCLE : Western Blot Assessment of Selected OXPHOS Subunits from Complexes I-V



Error bars = SEM; Triplicate determinations  
Data for each Complex I-V is expressed as percentage of the reference interval mean. 5% and 95% reference intervals are shown (upper and lower red lines).

## Summary

1. Assessment of OXPHOS subunits by Western blot is an essential component of patient diagnosis.
2. This testing can detect OXPHOS defects that are not evident by OXPHOS enzymology (3).
3. However, Western blot can be normal even in patients with identifiable mtDNA mutations (4-5). Hence, as reflected in the diagnostic criteria for mitochondrial disease, a multifaceted evaluation approach is essential to proper patient diagnosis (6). In most cases, no single test is diagnostic of mitochondrial disease.
4. Pathogenic mutations affecting polypeptide subunits of Complexes I, III and V can produce reductions in various OXPHOS enzyme subunits. This finding may be due to increased instability and degradation of the OXPHOS enzyme complex. This conclusion is consistent with analysis of supercomplex assembly and monomeric enzyme assembly.
5. MtDNA mutations affecting mitochondrial protein synthesis most commonly produces a decrease in OXPHOS enzyme subunits.
6. In some patients, OXPHOS enzyme subunits are increased. When assessed in the context of other OXPHOS testing (data not shown), these increases likely represent increased expression of OXPHOS enzymes and/or increases in mitochondrial mass.

## Conclusions

1. Shoffner, J. M., Lott, M. T., Lezza, A. M., Seibel, P., Ballinger, S. W., and Wallace, D. C. (1990) Myoclonic epilepsy and ragged-red fiber disease (MERRF) is associated with a mitochondrial DNA tRNA(Lys) mutation, *Cell* 61, 931-937.
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5. Byrne, E., Trounce, I., Marzuki, S., Dennett, X., Berkovic, S. F., Davis, S., Tanaka, M., and Ozawa, T. (1991) Functional respiratory chain studies in mitochondrial cytopathies. Support for mitochondrial DNA heteroplasmy in myoclonus epilepsy and ragged red fibers (MERRF) syndrome, *Acta Neuropathol* 81, 318-323.
6. Shoffner, J. M. (2008) Mitochondrial Diseases, In *MedLink Neurology* (Gilman, S., Ed.), MedLink Corporation, San Diego.