

Relationship Between Metabolic Rate Measurements, Mitochondrial Protein Chemistry, and Mitochondrial DNA (mtDNA) Mutations [P01.030]

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Background

MtDNA mutations have diverse effects on mitochondrial proteins including decreased stability of OXPHOS enzyme monomers and supercomplexes. Cellular responses to OXPHOS defects vary over time and include increases or decreases in OXPHOS proteins. Since about 95% of oxygen is consumed by OXPHOS, the resting metabolic rate (RMR) can assess whether oxygen utilization is increased such as with poorly coupled mitochondria or decreased such as when respiratory chain impairment is severe. Here we demonstrate that RMR measurements are an important tool in the investigation and clinical follow up of mitochondrial disease patients.

Methods

Retrospective analysis: RMR was determined in 7 patients with pathogenic mtDNA mutations and compared to the RMR from 10 patients with Leigh disease. Analysis of selected nuclear or mtDNA coded OXPHOS subunits from Complexes I-IV was performed by Western blot. OXPHOS enzyme activity, mtDNA copy number, and CoQ10 levels in muscle were compared.

Mitochondrial Disease Diagnosis Criteria (Table 1)

- 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).
- The activity measurements obtained from OXPHOS enzymology 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).
- Assessment of OXPHOS subunits by Western blot is an essential component of patient diagnosis. This testing can detect OXPHOS defects that are not evident by OXPHOS enzymology (3). Alternatively, 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).

*NOTE: for Table 1 mtDNA mutations, the term 'definite' is used to mean that the mutation meets criteria for a disease-causing mutation. 'Provisional' is used to describe mutations for which additional data is required to confirm or refute pathogenicity.

TABLE 1. MITOCHONDRIAL DISEASE DIAGNOSIS REQUIRES A MULTIFACETED APPROACH

CLINICAL	GENETIC		BIOCHEMISTRY & ENZYMOLOGY		
Phenotype	mtDNA Mutation*	mtDNA copy number	OXPHOS Complex Activity	OXPHOS Protein subunits	CoQ10 level in muscle tissue
Leigh Disease	NORMAL mtDNA	ABNORMAL INCREASED	ABNORMAL	ABNORMAL	NORMAL
Microcephaly, Retinitis pigmentosa	ATP6 8573 G>A definite	NORMAL	ABNORMAL	ABNORMAL	ABNORMAL
Leigh Disease	ATP6 9035 T>C definite	NORMAL	ABNORMAL	NORMAL	NORMAL
Leigh Disease	NORMAL mtDNA	NORMAL	ABNORMAL	NORMAL	NORMAL
Mitochondrial Encephalopathy, Lactic Acidosis, and Stroke-like episodes (MELAS)	Cyt b 15635 T>C definite	NORMAL	ABNORMAL	NORMAL	no data
Myopathy	16S rRNA 2083 T>C provisional	no data	ABNORMAL	ABNORMAL	no data
Leigh Disease	ATP6 8993 T>C definite	NORMAL	ABNORMAL	NORMAL	NORMAL
Leigh Disease	ND5 13514 A>G definite	NORMAL	ABNORMAL	ABNORMAL	NORMAL
Fatigue, myalgias	tRNA ^{Leucine} (UUR) 3280 A>G definite	NORMAL	ABNORMAL	ABNORMAL	NORMAL
Leigh Disease	NORMAL mtDNA	NORMAL	NORMAL	ABNORMAL	NORMAL
Chronic Progressive External Ophthalmoplegia (CPEO)	tRNA ^{Asparagine} 5670 A>G definite	NORMAL	ABNORMAL	ABNORMAL	NORMAL
Leigh Disease	NORMAL mtDNA	NORMAL	ABNORMAL	ABNORMAL	NORMAL
Fatigue, myopathy	Cyt b 9bp deletion definite	ABNORMAL INCREASED	ABNORMAL	ABNORMAL	NORMAL
Leigh Disease	ND6 14430 A>G provisional	NORMAL	ABNORMAL	ABNORMAL	NORMAL
Intractable seizures	ND4 11778 G>A definite	no data	ABNORMAL	ABNORMAL	no data
Leigh Disease	tRNA ^{Glycine} 10044 A>G provisional	no data	NORMAL	ABNORMAL	no data
Leigh Disease	ATP8 8504 T>C provisional	NORMAL	ABNORMAL	ABNORMAL	NORMAL

TABLE 2. TISSUE OXYGEN UTILIZATION IS INCREASED OR DECREASED IN MITOCHONDRIAL DISEASE

(% of predicted O ₂ utilization according to age and weight)
DECREASED <80%
29%*
34%
51%
56%
62%
71%
79%
NORMAL 80-120%
81%
86%
106%
108%
120%
INCREASED >120%
128%
130%
198%
209%
233%

RMR measurements in Mitochondrial Disease (Table 2)

- In this study, 5 patients had normal RMR in the 80-120% range, 7 had decreased RMR <80%, and 5 had increased RMR >120%.
- Of the patients with increased RMR, three with the most significant increases (198%, 209%, and 233% of predicted) had diverse mtDNA mutations in ND4, tRNA^{Glycine}, or ATP8, respectively.
- Of the patients with decreased RMR, five had the most significant decreases (29-62% of predicted) including three with mtDNA mutations in ATP6 or Cytochrome b.
- Factors such as decreased lean body mass and brain disease do not account for all RMR changes observed since patients with similar diseases can have significantly increased or decreased RMR values.
- RMR measurements are highly reproducible with low inter-measurement variance of approximately 2-4%.
- Patients are quiet/still during the RMR test and changes are NOT due to movement.
- RMR values shown here are in % of predicted Oxygen consumption taking age, gender, height and weight into account:
 $\% \text{ of predicted} = \frac{\text{actual RMR measured (kcal/day)}}{\text{predicted RMR (kcal/day)}}$

* NOTE: in one patient with Leigh Disease, an RMR increase from 29% to 99% was observed with CoQ10 treatment (800mg/day), suggesting either an improvement in OXPHOS function or possible uncoupling with increased oxygen utilization.

Summary

- Mitochondrial disease diagnosis requires a multifaceted approach. OXPHOS enzymology alone is not sufficient for diagnosis (6).
- RMR is an important tool in the investigation and clinical follow up of mitochondrial disease patients. Patients with OXPHOS defects may show increased resting metabolic rates reflecting an increased oxygen consumption to compensate for reduced ATP synthesis (likely due to uncoupling) or decreased resting metabolic rates reflecting decreased oxygen utilization by tissue mitochondria (6).
- The most severe decreases in RMR were observed in patients harboring mtDNA mutations affecting Complex V (ATP6) function.

Selected References

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