

## 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. 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.

Mutations in the adenine nucleotide translocase gene (ANT1) are associated with multiple mtDNA deletions and autosomal dominant forms of progressive external ophthalmoplegia (adPEO) (1,2). As a homodimer, ANT1 forms a pore (gated channel) in the inner mitochondrial membrane to allow passage of ADP into the matrix and ATP into the cytoplasm. ANT regulates the adenine nucleotide concentrations in the cytoplasm and within the mitochondria and mediates signals of nucleocytoplasmic energy consumption to the mitochondrial respiratory chain (3). Dysregulation of these channels by mutations in ANT1 result in instability of the mtDNA and accumulation of multiple mtDNA deletions.

The effects of a dominantly transmitted ANT1 (SLC25A4) mutation (Exon 2: Aspartate 104 Glycine) on clinical, biochemical, enzymologic, and protein chemistry testing are investigated.

## Clinical Description

**Clinical:**  
Chronic progressive external ophthalmoplegia  
Fatigue

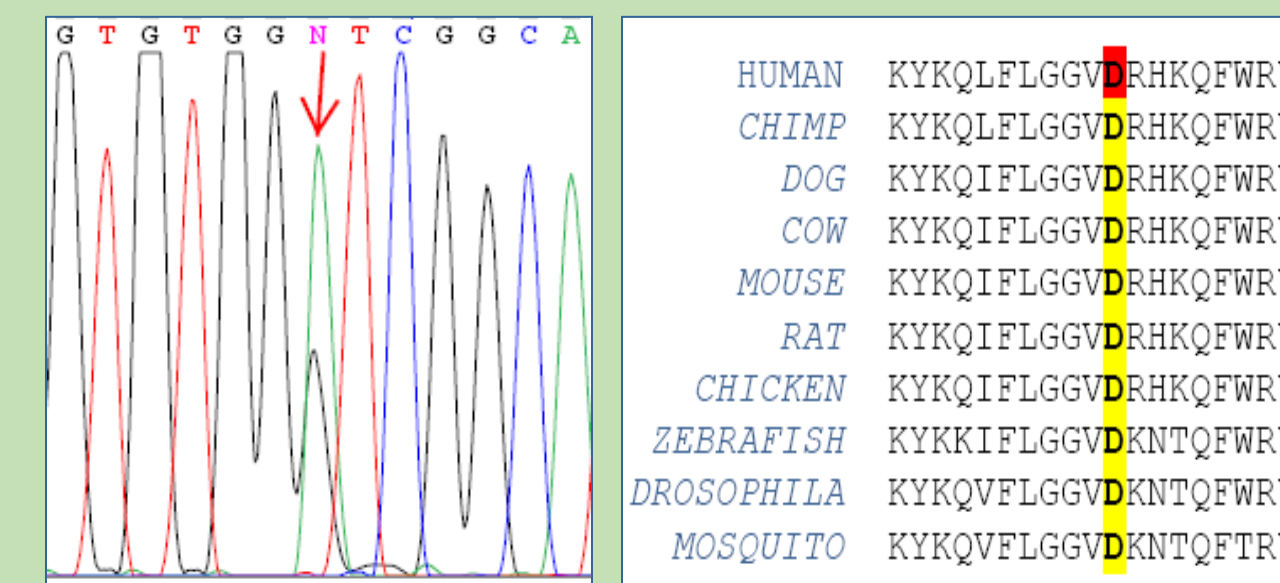
**Family History:** affected mother; unaffected son  
•Myopathy  
•Ptosis  
•Eye muscle dysfunction

**Laboratory:**  
Abnormal histochemistry: ragged-red fiber myopathy  
Fibroblast fatty acid oxidation: increased medium & long chain acylcarnitines in a pattern consistent with that observed in patients with OXPHOS defects

NOTE: Quantitation of mtDNA in muscle and mtDNA Southern blot for detection of deletions were normal

## Heterozygous ANT1 gene Mutation: (Adenine Nucleotide Translocase) Aspartate 104 Glycine (D104G)

Pathogenic ANT1 mutation D104G (4) segregates with disease in family (found in patient and mother; not in unaffected son).



## Abnormal 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	2.34
Net Routine Flux Control Ratio	0.32-0.44	0.32	0.35	0.37	0.40	0.43	0.43
Respiratory Control Ratio	7.60-14.88	7.10	9.28	10.79	12.30	14.5	11.82
Leak Flux Control Ratio	0.07-0.13	0.06	0.08	0.10	0.11	0.13	0.09
Phosphorylation Respiratory Control Ratio	0.22-0.36	0.22	0.25	0.28	0.30	0.34	0.34

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 Subunit Protein Chemistry: Complex I & III defects

OXPHOS Enzyme Subunit Tested (Normalized to GAPDH)	Patient	Mean [5%-95%]
Complex I (ND6 subunit; mtDNA coded)	41% [5-25% level]	67.0% [38.2-95.8%]
Complex II (30 kDa subunit; nuclear DNA coded)	26%	40.4% [19.5-61.2%]
Complex III (core 2 subunit; nuclear DNA coded)	56% [5-25% level]	75.6% [50.9-100.3%]
Complex IV (COX II subunit; mtDNA coded)	98%	111.3% [73.5-149.0%]
Complex V (F1 alpha subunit; nuclear DNA coded)	77%	94.5% [65.4-123.6%]

The Complex I (ND6) and Complex III (Core 2) subunits were at the lower limits of the reference ranges. There is significant overlap between normals and abnormals below the 25% level.

## Muscle (OXPHOS) Enzymology: Complex I & III defects

OXPHOS Enzyme Assay (Activity is expressed as nanomoles substrate/minute/mg of mitochondrial protein)	Patient Activity	Mean±SD [5%-95%]
Complex I (n-decyl CoQ)	4 (<5% level)	85 ± 34 [47-160]
Complex I (CoQ1)	106 (5-25% level)	246 ± 118 [97-438]
Complex I+III	134 (5-25% level)	329 ± 198 [117-698]
Complex II+III	420	407 ± 210 [146-846]
Complex III	935 (5-25% level)	1461 ± 473 [837-2368]
Complex IV (Freeze/thaw)	2894	2388 ± 916 [1049-4069]
Complex IV (sonicated)	3645	2717 ± 943 [1256-4458]

OXPHOS enzymology was performed on mitochondria isolated from fresh muscle. Defects were diagnosed by comparison of activity with the 5%-95% reference intervals calculated from 254 controls.

Complex I & III activities were at or below the lower limits of the reference ranges. There is significant overlap between normals and abnormals below the 25% level.

## Abnormal Exercise Testing

Decreased resting metabolic rate (RMR) exercise testing:

1. RMR value was 68% of predicted O<sub>2</sub> utilization which indicates respiratory chain impairment.
2. Anaerobic Threshold: Low (11.7 ml/kg/min)
3. HR Response: Low peak HR (73% age predicted) with a curvilinear pattern.
4. BP Response: Rest: Normal. Peak: Normal.
5. Oxygen Pulse: Low peak O<sub>2</sub>-pulse. Trend: Plateau during last 2.5 min of exercise.
6. EKG: Unremarkable.
7. Cardiovascular status: Myocardial dysfunction: the decreasing stroke volume with increasing work rate is consistent with exercise-induced myocardial dysfunction during the last 2.5 min of exercise.

## Summary

1. Autosomal dominant mutation of the ANT1 (SLC25A4) gene causes an instability in the mtDNA resulting in accumulation of multiple mtDNA deletions. ANT1 encodes the heart/skeletal muscle isoform of the adenine nucleotide translocator 1.
2. OXPHOS Western blot of selected subunits revealed a decrease in the mtDNA-encoded Complex I ND6 subunit and the nuclear DNA-encoded Complex III core 2 subunit.
3. OXPHOS enzymology was consistent with the Western blot data. Decreased presence of Complex I & III subunits correlates with observed decreases in enzyme activity measurements of Complex I and Complex III. This data is also consistent with the abnormalities observed with high resolution respirometry.
4. No single test is sufficiently sensitive or specific for mitochondrial disease diagnosis, a carefully performed, multi-faceted approach is required for appropriate diagnosis.

## Selected References

1. Van Goethem, G., et al. 2002. Progressive external ophthalmoplegia and multiple mitochondrial DNA deletions. *Acta Neurol Belg.* 102(1):39-42.
2. Chen, X. 2002. Induction of an unregulated channel by mutations in adenine nucleotide translocase suggests an explanation for human ophthalmoplegia. *Hum Molec Genet.* 11:1835-43.
3. Kaukonen, J., et al. 2000. Role of Adenine Nucleotide Translocator 1 in mtDNA Maintenance. *Science* 289:782-785.
4. Komaki, H., et al. 2002. A novel D104G mutation in the adenine nucleotide translocator 1 gene in autosomal dominant progressive external ophthalmoplegia patients with mitochondrial DNA with multiple deletions. *Ann. Neurol.* 51: 645-648.