Lack of aprataxin impairs mitochondrial functions via downregulation of the APE1/NRF1/NRF2 pathway

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Introduction

Ataxia oculomotorapraxia type 1 (AOA1) is an autosomal recessive disease characterized by early-onset cerebellar ataxia with oculomotor apraxia, cortico-spinal tracts involvement, peripheral neuropathy and mental retardation.
AOA1 is caused by mutations in *APTX*, which encodes the DNA strand-break repair protein aprataxin (APTX)
APTX has a role in mitochondrial function:

- APTX has been localized in mitochondria,
- APTX depletion in human cell lines, results in reduced mitochondrial mass, mtDNA damage and decreased mtDNA copy number,
- Reduced Coenzyme Q_{10} has been observed in muscle and fibroblasts of patients with AOA1 carrying a specific APTX mutation (p.W279X)

<u>Mechanism underling mitochondrial alteration in AOA1 is still unclear</u>

The aim of the study is to determine the role of APTX in mitochondrial metabolism disruption

Results

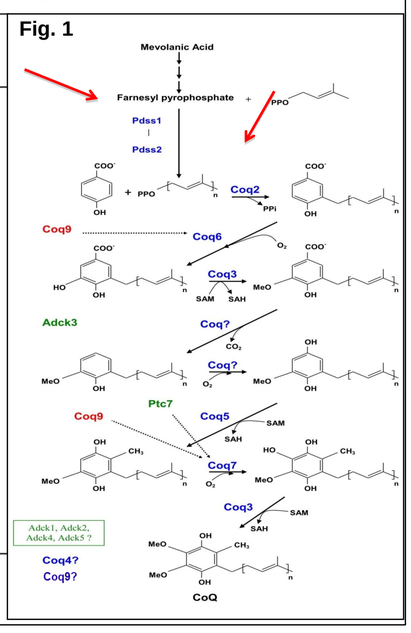
APTX-mutant fibroblasts show reduced levels and biosynthesis of CoQ_{10}

CoQ_{10} was measured by HPLC in primary fibroblasts derived from 9 patients. Seven out of nine patients had reduced level of CoQ10 (Table 1). To define the cause of CoQ_{10} deficiency, we studied the biosynthesis of CoQ_{10} in fibroblasts, using the specific substrate (^{14}C-PHB) of the condensation of the decaprenyl tail (Fig. 1). Reduction in the rate of ^{14}C-PHB incorporation into the CoQ_{10} molecule suggested a defect in COQ1 (PDSS1 and PDSS2) enzyme.

Coenzyme Q_{10} Patient	Mutation	CoQ (ng/mg prot)	CoQ (%)	CoQ biosynthesis (%)
P1	pW279X/pW279X	43.5	68.2	62.3
P2	pW279X/pW279X	33.4	52.3	66.2
P3	pW279X/*	46.4	72.7	54.5
P4	pR306X/pR306X	95.1	149.0	n/a
P5	pW279X/pW279X	27.7	43.4	39.7
P6	pW279X/pW279X	61.9	97.0	n/a
P7	pW279X/pW279X	40.4	63.3	45.3
P8	pW279X/pQ181X	41.5	65.0	43.7
P9	pW279X/pW279X	46.6	73.0	38.6
Controls ($n=8$)		63.8 ± 4.3	100 ± 6.8	100 ± 6.6
Biosynthesis of CoQ_{10}				
Assay 1	CoQ_{10} synthesis	Percent of control mean		
Controls ($n=7$)	2945.4 ± 195 DPM/mg prot/day	100 ± 6.6		
Patients ($n=7$)	1796.3 ± 54 DPM/mg prot/day	61.0 ± 1.8		
Assay 2				
Controls ($n=5$)	62.8 ± 10.4 DPM/mg prot/h	100 ± 21.5		
Patients ($n=3$)	56.4 ± 3.1 DPM/mg prot/h	89.8 ± 4.9		

n/a, not available; DPM, disintegration per minute; prot, proteins.
Values are expressed as mean ± SEM.

Fig. 1



APTX-mutants show reduced PDSS1 expression

We investigated the steps of CoQ_{10} biosynthesis by measuring mRNA and protein levels of enzymes involved in the CoQ_{10} synthetic pathway and/or its regulation. Fibroblasts with the p.W279X mutation and low levels of CoQ_{10} showed significantly reduced mRNA expression and protein level of PDSS1 (Fig. 2). The same result was obtained in APTX depleted HeLa cells (Fig. 3).

Fig. 2

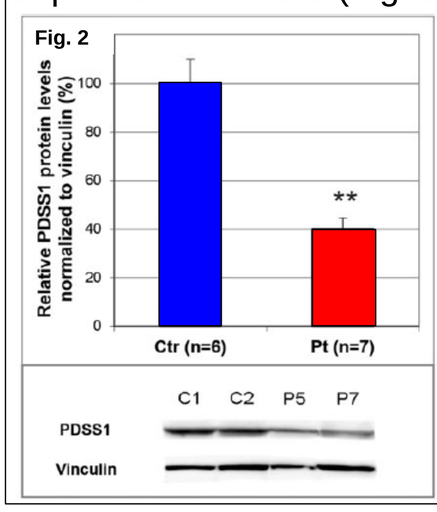
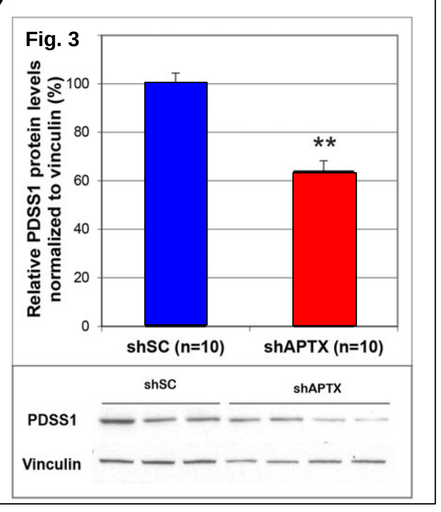


Fig. 3



APTX-mutants manifest reduced respiratory chain activity

In order to assess the metabolic consequences of the mitochondrial abnormalities observed, we assessed the energy metabolism in CoQ_{10}-deficient fibroblasts. ATP turnover, expressed as coupling efficiency, showed no differences between control and APTX-mutant fibroblasts. However, respiratory control ratio was reduced in AOA1 cells (62±19% of controls) relative to controls. We observed mild decrease in Complex I+III activity (61±7% of controls), consistent with the mild CoQ10 deficiency. SDH (Complex II) activity was significantly reduced (63 ± 9% of controls) (Fig. 5).

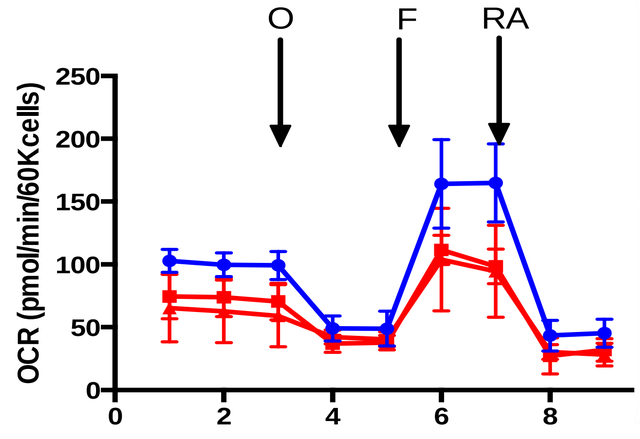
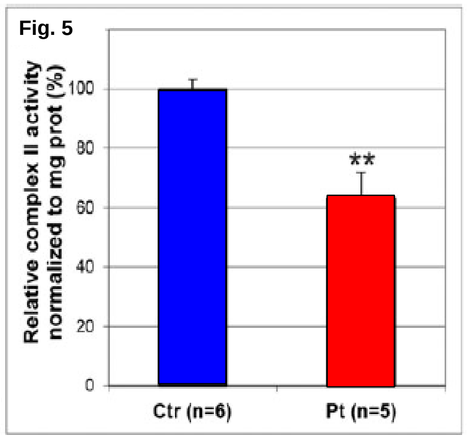


Fig. 5



APE1/NRF1/NRF2 pathway is altered in APTX-mutant cells

Multiple mitochondrial genes, including succinate dehydrogenase subunit A (SDHA) and PDSS1, are co-regulated by NRF1 and NRF2 (Fig. 6). Only APTX-mutant fibroblasts and APTX knockdown cells, showed decreased levels of both transcription factors NRF1 and NRF2 (Fig. 7), consistent with the mild reduction of CoQ_{10} and SDHA protein in these cells. APTX interacts with a variety of proteins; we analyzed the levels APE1, an interacting partner of aprataxin, known to regulate NRF1 function. AOA1 fibroblasts and APTX depleted HeLa cells showed decreased levels of APE1 (Fig. 7).

Fig. 6

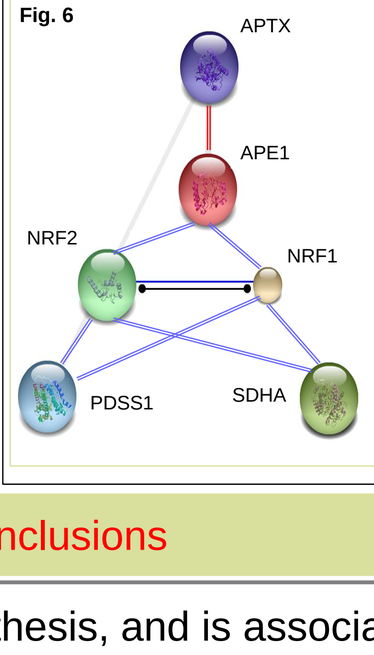
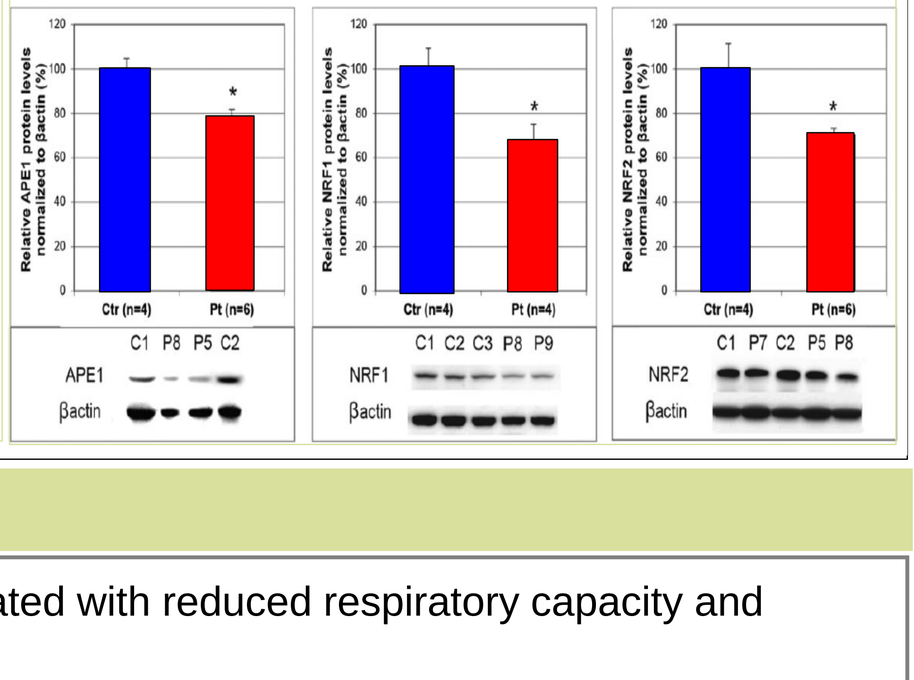


Fig. 7



Conclusions

CoQ10 deficiency in AOA1 is due to diminished CoQ_{10} biosynthesis, and is associated with reduced respiratory capacity and decreased mitochondrial respiratory chain enzymes.
We demonstrated that mitochondrial dysfunction in AOA1 is related to its role in transcription regulation through the APE1/NRFs pathway.
Our findings shed some light on the link between APTX and mitochondrial function, which may lead to better understanding of the pathogenesis of AOA1 and other degenerative disorders, giving the opportunity to identify targets for novel therapeutic approaches.

References

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