Degeneration and aging – the effect of pathogenic mitochondrial mutations

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Introduction

This project addresses the fundamental question of stochastic ageing due to mitochondrial DNA (mtDNA) mutations, and whether radical oxygen species (ROS) are involved in the process. Even though mitochondria have been shown to be a major site of ROS production and mitochondrial damage have strong association with ageing, no convincing evidence exists about the causal relationship of mtDNA mutations provoking ROS production, leading into DNA and protein damage, and thus producing cellular dysfunction and further ageing.

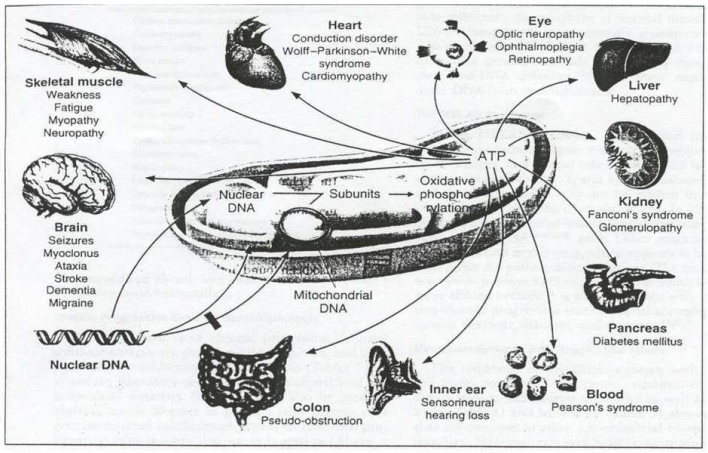

Fig.1 Mutations in human mitochondrial or nuclear genes affecting the mitochondrial energy production system cause diseases with degenerative changes in several different organs. (Johns 1995)

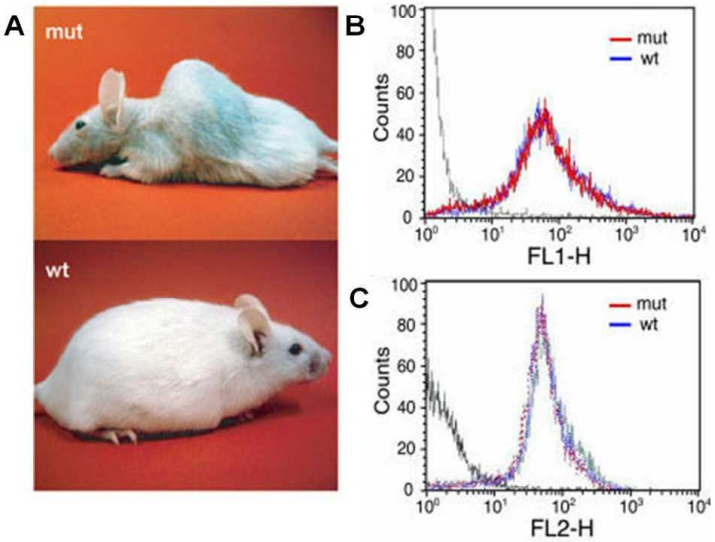

Fig.2 Mutator mouse model associates premature aging phenotype with mitochondrial mutations. Mice accumulating mutations in the mitochondrially encoded respiratory enzyme genes display reduced lifespan and premature onset of ageing-related phenotypes such as weight loss, reduced subcutaneous fat, alopecia (hair loss), kyphosis (curvature of the spine), osteoporosis, anaemia, reduced fertility and heart enlargement (A). (Trifunovic et al., 2004) However, ROS production was not increased in the form of hydrogen peroxide (B) or superoxide (C). (Trifunovic et al., 2005)

Table 1. Human diseases due to mtDNA mutations. Leber hereditary optic neuropathy (LHON) and mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS) as examples.

LHON	MELAS
-G11778A, G3460A, T14484C common mutations in complex I genes	-A3243G common mutation in $tRNA^{Leu}$ gene
-Painless loss of vision in both eyes during weeks to months	-Muscle and central nervous system affision with hemiparesis, cortical blindness, sensorineural deafness, endocrine dysfunctions etc
-Mild to moderate decline in complex I activity	-Complex I and complex IV deficiency

Hypothesis and aims

According to our hypothesis human pathogenic mtDNA mutations resulting in different clinical phenotypes cause divergent consequences in mitochondrial metabolism. To elucidate these changes and their contribution to longevity, the functional genetics approach will be used to reveal the consequences of biochemically characterized deleterious mitochondrial complex I mutations, modelled in *Escherichia coli* enzyme.

Results

Table 2. LHON mutations in ND6 subunit affect ubiquinone binding by lowering the binding affinity or introducing substrate inhibition. Kinetics of dNADH:DB reaction (K_m Michaelis Menten binding constant and K_s' substrate inhibition binding constant) and enzyme amount (HAR reductase). The values are means ± S.E.M. (Pätsi, Kervinen et al., 2008)

Mutant	Enzyme activity	Substrate affinity		Enzyme amount
		K_m (μM)	K_s' (μM)	
control	100%	23 ± 2	4227	100 %
Y59C – LHON 14498	73%	22 ± 2	594*	91 %
M64V – LHON 14484	83%	33 ± 4*	3409	219 %
M72V – LHON 14459	53%†	39 ± 4†	∞‡	100 %

*p<0.05, †p<0.01 when compared to control (HAR not tested).
‡No exact value could be calculated because of low affinity.

LHON

Table 3. MELAS position mutations decrease membrane bound complex I amount and complex I catalytic activity, but unlike the LHON mutations in the ND6 subunit, did not alter ubiquinone binding (not shown here). (Kervinen et al., 2006)

Mutant	Enzyme activity	Enzyme amount	
		E. coli	*P. denitrificans*
control	100 %	100 %	100 %
E228K – MELAS 3946	2 %	55 %	n.a.[1]
E228Q	3 %	42 %	13 %
Y229H – MELAS 3949	74 %	71 %	n.a.

[1]Not available

MELAS

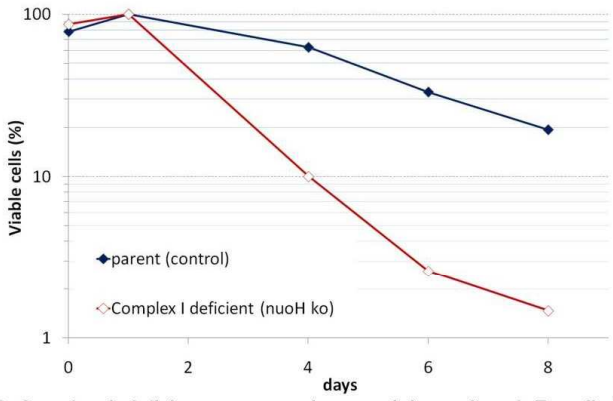

Fig.3 Complex I deficiency causes decreased longevity of *E. coli* during glucose starvation. The number of colony forming units of MG1655 (parent) and MG1655-*nuoH* (complex I deficient) *E. coli* strains is presented during incubation in 0.08 % glucose/M9 after the stop of growth.

Conclusions and future perspectives

The effects of MELAS and LHON disease mutations differ in the biochemical level. In addition, complex I enzyme was found important for the longevity of *E. coli* during carbon starvation. We are currently exploring the further consequences of these mutations and whether they affect longevity.

Kervinen, M. *et al.*, (2006). The MELAS mutations 3946 and 3949 perturb the critical structure in a conserved loop of the ND1 subunit of mitochondrial complex I. *Hum. Mol. Genet.* **15**, 2543-2552.

Pätsi, J., Kervinen, M. *et al.* (2008). Leber hereditary optic neuropathy mutations in the ND6 subunit of mitochondrial complex I affect ubiquinone reduction kinetics in a bacterial model of the enzyme. *Biochem. J.* **409**, 129-137.

Trifunovic, A. *et al.* (2004). Premature ageing in mice expressing defective mitochondrial DNA polymerase. *Nature* **429**, 417-423).

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