fibroblasts. The impairment of proton-translocation activity of COX was directly confirmed by mitochondrial membrane potential measurements using $TPP^+$ electrode. While proton pumping at complexes I and III in patient fibroblasts was similar to controls, ascorbate + TMPD substrates were unable to support generation of proton gradient. Consequently, mitochondrial membrane potential as estimated by JC-1 staining was lower in intact patient fibroblasts, leading to extremely decreased rates of mitochondrial ATP production to 25% of control values. Such drop in energy provision ultimately resulted in two-fold decrease of ATP/ADP ratio in patient cells grown in galactose medium, when most of ATP must be synthesized by mitochondria. In contrast to profound impairment of mitochondrial energetics, no changes in the production of reactive oxygen species (ROS) or antioxidant defences could be found in patient fibroblasts. This is perhaps due to decreased mitochondrial membrane potential, which may serve as a paradoxical ROS-preventing mechanism. We conclude that unlike to mitochondrial disorders caused by dysfunction of ATPase or complex I, the pathogenic mechanism of COX deficiencies seems to have only single component — impaired mitochondrial energy provision.

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### 4P.11 Increased oxidative stress in fibroblasts from patients with ATP synthase deficiency

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Genetic defects in enzymes of oxidative phosphorylation cause a broad spectrum of mitochondrial encephalomyopathies. Apart from diminished ATP production per se, elevated oxidative stress is implicated in pathogenic mechanism of mitochondrial diseases. In our work we used fibroblasts from patients with isolated deficiency of ATP synthase caused by mutation in *TMEM70* gene to study consequences on mitochondrial function, *in vivo* ROS production and levels of cellular ROS scavengers. With the aim to elucidate how the low ATP synthase content affects mitochondrial energy provision, we have investigated fibroblasts from patients with ATP synthase content decreased to <30% of the control. Measurements of cellular respiration showed insufficient ATP synthase capacity for basal respiration and mitochondrial ATP synthesis was decreased to 26–33%.Cytofluorometric analysis using TMRM revealed increased mitochondrial membrane potential ($\Delta\psi_m$) at state 3-ADP in patient cells. Consequently, viability of patient fibroblasts was more sensitive to ATP synthase inhibitors oligomycin or aurovertin. Analysis of ROS production by CM-$H_2$DCFDA demonstrated increase in ROS production and decrease of MnSOD activity in two patients, while level of main cellular ROS scavenger glutathione was only mildly decreased compared to control. In the third patient ROS production was not changed but MnSOD activity was dramatically increased and glutathione level decreased. Our results indicate two-component pathological mechanisms in ATP synthase deficient patient cells — impairment of ATP provision and oxidative stress.

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### 4P.12 *POLG* mutations lead to decreased mitochondrial DNA repopulation rates after EtBr-induced depletion in fibroblasts

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Mutations in nuclear genes encoding proteins that are involved in mitochondrial DNA (mtDNA) maintenance, e.g. *POLG, TK2*, are associated with various neurodegenerative disorders [1]. All pathogenic mutations in these nuclear genes lead to mtDNA depletion and secondary mtDNA mutations, which cause dysfunction of the oxidative phosphorylation and lead to disease phenotype. Until now it is a major challenge to demonstrate the direct functional consequences of those mutations. To address the issue, whether *POLG* or *TK2* mutations lead to impaired mtDNA maintenance, a kinetic assay for mtDNA replication in primary human fibroblasts was performed. Different fibroblast cell lines were depleted of their mtDNA by treatment with ethidium bromide (EtBr) and the rates of mtDNA repopulation were determined. Here we demonstrate that the rate of mtDNA depletion, induced by EtBr, showed no significant difference between patients and controls. In contrast, the restoration of mtDNA levels is significantly delayed in fibroblasts from patients with *POLG* mutations, while TK2 mutations have no effect on mtDNA repopulation rates. These findings provide the first *in vivo* evidence that pathogenic *POLG* mutations directly influence the mtDNA maintenance in human cells. Furthermore, these results are in line with *in vitro* data showing reduced catalytic activity and processivity for several pathogenic *POLG* alleles [2–5].

**References**

[1] DiMauro S. et al. (2005) *Ann. Med.* **37:** 222-232.
[2] Chan S.S. *et al.* (2006) *Hum. Mol. Genet.* **15:** 3473-3483.
[3] Graziewicz M.A. (2004) *Nat. Struct. Mol. Biol.* **11:** 770-776.
[4] Chan S.S. *et al* (2005) *J. Biol. Chem.* **280:** 31341-31346.
[5] Longley M.J. *et al.* (2005) *Gene.* 125-131.

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### 4P.13 Impact of diabetes-associated lipoproteins on oxygen consumption, enzymatic activities of mitochondrial respiratory chain complexes

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Diabetes is a mitochondrial disease. Atherosclerotic coronary artery disease (CAD) is the leading cause of mortality in diabetic patients. Mitochondrial dysfunction and increased production of reactive oxygen species (ROS) are associated with diabetes and CAD. Elevated levels of glycated low density lipoproteins (glyLDL) and oxidized LDL (oxLDL) were detected in patients with diabetes. Our previous studies demonstrated that oxLDL and glyLDL increased the generation of ROS and altered the activities of antioxidant enzymes in vascular endothelial cells (EC). The present study examined the effects of glyLDL and oxLDL on oxygen consumption in mitochondria and the activities of key enzymes in mitochondrial electron transport chain (ETC) in cultured porcine aortic EC. The results demonstrated that glyLDL or oxLDL significantly impaired oxygen consumption in Complex I, II/III and IV of mitochondrial ETC in EC compared to LDL or vehicle control detected using oxygraphy. Incubation with glyLDL or oxLDL significantly reduced mitochondrial membrane potential, the levels of $NAD^+$/NADH ratio, and the activities of mitochondrial ETC enzymes (NADH–ubiquinone dehydrogenase, succinate cytochrome *c*

reductase, ubiquinone cytochrome *c* reductase, and cytochrome *c* oxidase) in EC compared to LDL or control. The abundance of mitochondria-associated ROS and the release of ROS from EC were significantly increased following glyLDL or oxLDL treatment. The findings suggest that glyLDL attenuates activities of key enzymes in multiple mitochondrial ETC, decreases mitochondrial oxygen consumption and mitochondrial membrane potential, and increases ROS generation from mitochondria in EC, which potentially contributes to mitochondrial dysfunction and vascular disorders in diabetic patients.

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### 4P.14 Cell biological consequences of isolated complex I deficiency in a KO mouse model

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In humans, deficiency of complex I (CI or NADH:ubiquinone oxidoreductase) is associated with a wide range of progressive neurological disorders that are characterized by an early onset and a short devastating course. CI resides in the mitochondrial inner membrane (MIM), where it channels electrons from food-derived NADH into the electron transport chain (ETC). Together with three other complexes (CII-CIV) and the ATP-generating enzyme $F_OF_1$-ATPase (CV), CI constitutes the mitochondrial oxidative phosphorylation system (OXPHOS). CI is a large (about 1 MDa) multi-subunit complex that consists of 45 different subunits of which 7 are encoded by the mitochondrial DNA (mtDNA) and the remainder by the nuclear DNA (nDNA). To study the pathophysiology of CI dysfunction, we use a novel knockout (KO) mouse model lacking an nDNA-encoded CI subunit (NDUFS4), which is a mutational hotspot in human CI deficiency. For this purpose we generated mouse embryonic fibroblast (MEF) cell lines from wild type (WT) and KO animals. Analysis of mitochondrial fractions revealed a greatly reduced CI activity in KO MEFs (about 5% of WT), whereas other activities (CII, CIII, CIV, CV and citrate synthase) were normal. At the protein level, MEFs from KO animals lacked fully assembled CI but instead contained a CI subcomplex of about 850 kDa. Reintroduction of the NDUFS4 subunit in KO cells led to reappearance of the fully assembled CI holocomplex. Functionally, KO MEFs contained mitochondria with a hyperpolarized membrane potential, probably due to reverse-mode action of CV. When cultured in glucose-containing (GLU) medium, reactive oxygen species (ROS) levels in WT and KO cells were identical. However, when a galactose (GAL) medium was used, KO MEFs displayed increased ROS levels whereas WT MEFs did not. In KO MEFs, complementation of the *NDUFS4* gene defect fully normalized the increased ROS levels. NADP(H) levels in KO MEFs were higher than in WT MEFs, whereas cytosolic resting [ATP] (determined with the novel fluorescent ATP-sensor 'ATeam') where the same. Currently, experiments are in progress to determine the effects of NDUFS4 knockout on cytosolic calcium handling and expression of energy-sensing proteins. Moreover, established antioxidants and nutraceuticals are tested for their beneficial action on WT and KO MEFs.

**References**

[1] P. Willems (2008) *Cell Calcium* **44**: 123–133.
[2] F. Valsecchi *et al.* (2009) *BBA Molecular Basis of Disease* **1792:** 1130–1137.
[3] Koopman W *et al.* (2010) *Antioxidants and Redox Signaling* **12**: 1431–1470.

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### 4P.15 Molecular investigation of riboflavin-responsive multiple acyl-CoA dehydrogenase deficiency (RR-MAD) patients

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Riboflavin-responsive multiple acyl-CoA dehydrogenase deficiency (RR-MAD) is a lipid storage myopathy characterised by muscle weakness, decreased β-oxidation and muscle carnitine, altered urinary organic acids and plasma acyl-carnitine. We studied a cohort of 11 RR-MAD patients, performing a battery of biochemical and molecular tests to better characterise the metabolic disturbance of this disease and to explore the pathophysiological events [1–3]. Biochemical alterations of pre-therapy patient muscles included: (i) reduction in β-oxidation and in flavin-dependent enzymes (short and medium-chain acyl coenzyme A dehydrogenases, complexes I and II); (ii) reduction of FMN and FAD concentrations, (iii) increased activity of mitochondrial FAD pyrophosphatase [1, 3], thus suggesting an altered regulation of mitochondrial flavin cofactor homeostasis [4]. Proteomic investigation of muscle mitochondria in one RR-MAD patient [3] revealed decrease or absence of several proteins. All these deficiencies were completely rescued after riboflavin treatment. Proteomic data indicate that the enzymatic defects are multiple, coordinated, riboflavin-responsive and more extensive than previously described. In patients with RR-MAD the molecular defect is still unknown. Recently it was described that RR-MAD is associated with defects in electron transfer flavoprotein dehydrogenase (*ETFDH)* gene in a large proportion of cases. Therefore we performed molecular investigation by sequencing the *ETFDH* gene to identify the defects in this group of RR-MAD patients. The genomic sequence of each exon of *ETFDH,* including intron/exon boundaries has been PCR-amplified for 30 cycles. The PCR products have been purified and directly sequenced.

**References**

[1] Vergani Brain 122 1999 2401–2411.
[2] Russell et al J. Clin. Endocrinol. Metab. 88 2003 5921–5926.
[3] Gianazza Electrophoresis 27 2006 1182 1198.
[4] Barile et al Eur. J. Biochem. 249 1997 777–785.
[5] R.K.J. Olsen et al Brain 130 2007 2045–2054.
[6] Y. Yotsumoto et al Mol. Genet. Metab. 94 2008 61–67.

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