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_2DCFDA 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].

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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*