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4P.5 Respiratory chain protein analysis, gene expression profiles of fibroblast cell lines from 9 patients with *SURF1* gene mutations

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Isolated deficiency of cytochrome *c* oxidase (COX) is most frequently caused by mutations in *SURF1* gene and manifest as fatal Leigh syndrome. Exact function of Surf1 protein (Surf1p) is still unknown but it may be involved in an early step of assembly during the association of CoxII subunit with CoxI–CoxIV–CoxVa subassembly. Absence of Surf1p leads to decreased content and activities of COX, accumulation of COX assembly intermediates and decrease of mitochondrial membrane potential. The aim of study was to describe how *SURF1* mutations influence protein and transcript level of OXPHOS genes and if there are specific changes in other non-mitochondrial genes. For experiments were used cell fibroblast lines of 9 patients with *SURF1* mutations and of 5 controls. Protein levels in cell homogenates and in isolated mitochondria were analysed by SDS-PAGE and 2D BN/SDS-PAGE combined with immunobloting using specific antibodies to subunits of the respiratory chain complexes (RCC). Expression data were obtained using Agilent human whole genome array 44K. Analysis of COX subunits revealed similar changes in the content of CoxI, CoxII, CoxIII and CoxIV in patient cells and mitochondria that were decreased to 13%–50% of controls while the CoxVa was less affected, 63% of controls. 2D analysis revealed accumulated CoxVa in the form of unassembled monomer or CoxVa–CoxIV heterodimer but neither of these subunits were present in 80 kDa intermediate containing CoxI. In response to COX deficiency both the cellular and mitochondrial content of RCC I and III was increased to 130% and 142% of controls. Expression profiles did not reveal significant and consistent changes in mRNA levels of OXPHOS subunit genes or pro-mitochondrial regulatory genes such as *PGC1A*, *NRF1* or *TFAM*. Our study indicates that observed compensatory changes result from posttranscriptional regulation.

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4P.6 Molecular studies of Polish patients with respiratory chain complex I deficiency

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Complex I (NADH:ubiquinone oxidoreductase, CI) is the largest, the most complex and the most crucial of the five multisubunit enzymes which belong to the OXPHOS system located in the inner mitochondrial membrane. The function of CI is to transfer electrons from NADH to ubiquinone, a process during which proton force is generated to enable ATP synthesis. NADH:ubiquinone oxidoreductase is composed of 46 protein subunits, which belong either to flavoprotein fraction, iron–sulphur fraction or hydrophobic fraction. Seven of these subunits are encoded by mitochondrial genes, with the remaining ones being encoded by nuclear genes. The highest level of their expression in humans is observed in brain, heart, skeletal muscles, kidneys and liver. Mutations in complex I subunits are associated with CI activity and a wide spectrum of mitochondrial disorders. Being responsible for 30% of all respiratory chain disorders in humans, this particular syndrome is inherited in autosomal recessive manner or it may be chromosome X-linked. The following genes: (1) mitochondrial genes: *MTND1, MTND2, MTND3, MTND4, MTND4L, MTND5* and *MTND6*; (2) nuclear genes: *NDUFS1, NDUFS2, NDUFS3, NDUFS4, NDUFS6, NDUFS7, NDUFS8, NDUFV1* and *NDUFV2* have been selected and analysed. All these genes are characterised by the same criteria. Firstly, they play the most important role in proper functioning of complex I. Secondly, they are highly conserved in the course of evolution. Finally, 55 different mutations have already been found in them (including mononucleotide substitutions, deletions, duplication, and inversion), mutations which cause such diseases as Leigh syndrome (LS), LHON, MELAS, Alzheimer disease and Parkinson disease. We present the results of molecular analysis of 18 Polish patients, with clinically and biochemically confirmed CI deficiency. The experiments involved three stages: isolation of cDNA from fibroblasts or genomic DNA from muscle biopsies and/or blood; PCR analysis; direct sequencing. In one patient m.3697G>A mutation, associated with mitochondrial cytopathy, was found in *MTND1* gene. In other 5 patients with LS 3 different mtDNA mutations were found: m.10191T>C (*MTND3*), m.13513G>A (*MTND5*), and m.14487T>C (*MTND6*). Additionally three polymorphic variants were observed in two other patients: p.V4V, p.G66G (*NDUFS4*) and p.A280V (*NDUFS2*).

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4P.7 Modeling in yeast the pathogenic T8851C mutation of human mtDNA reveals an ATP synthase with aberrant catalytic properties, defective mitochondrial shaping

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De Meirleir *et al.* (*Pediatr. Neurol.* 13: 242–246, 1995) reported a 2.5-year-old boy with bilateral striatal lesions presumed to be the