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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 result of a point mutation (T to C) at nucleotide position 8851 of the mitochondrial DNA (mtDNA). This mutation changes a highly conserved tryptophane residue to arginine at amino acid position 109 of the subunit *a* of mitochondrial F_1F_O-ATP synthase, a complex which provides most of the cellular ATP in humans. Nothing is known on the consequences of the T8851C mutation on the mitochondrial ATP synthase. To gain insight into the primary pathogenic mechanisms induced by T8851C, we have investigated the consequences of this mutation on the ATP synthase of yeast where the protein homologous to subunit *a* (referred to as Atp6p) is also encoded by the mtDNA. The modified yeast exhibited a very slow growth phenotype on non-fermentable carbon sources, both at 28 °C (the optimal temperature for growing yeast) and at 36 °C. In *vitro*, mitochondria from T8851C yeast grown at 28 °C showed a 60% deficit in ATP production, while those prepared from the mutant grown at 36 °C had an ATP synthesis activity below 5% that of the wild type. The mutated F_1F_O complex was correctly assembled, at both temperatures, and had a very poor ATPase activity (10% that of the wild type), both in mitochondria and after purification. Electron microscopy revealed that many of the mitochondrial matrices in T8851C yeast grown at 36 °C exhibited septae made of apposed inner mitochondrial membranes. Another anomaly was an increased mitophagic activity, presumably in response to the T8851C-induced damaging of mitochondria. Thus, in addition to a bioenergetic deficit, alterations in mitochondrial dynamics and homeostasis may also participate in the pathogenic mechanism induced by T8851C.

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4P.8 Iron deficiency in children with mitochondrial disease

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Mitochondrial disease is an energy metabolic disorder with various organ involvements. Iron is widely known to be one of the most important nutriments required for normal brain development and several essential metabolic functions. We retrospectively studied the laboratory data on iron deficiency (ID) in 69 children with mitochondrial respiratory chain complex (MRC) defects by biochemical enzyme assay using muscle tissue. We analyzed the differences between groups of mitochondrial disease based on the presence of ID. ID has higher prevalence in children with mitochondrial disease than in the normal population. There were 6 (9%) patients with low hemoglobin, 12 (17%) with low serum ferritin, and 22 (32%) with low transferrin saturation levels among children with MRC defects. In comparisons between the ID and the non-ID group of MRC-defect patients, the frequency of MRC I defect was significantly higher in the ID group while that of MRC IV defect was higher in the non-ID group. Abnormal brain magnetic resonance imaging (MRI) findings were more frequently detected in the ID group. The incidence of failure to thrive and gastrointestinal symptoms were significantly higher in the ID group. Early diagnosis and proper treatment of ID are recommended. Especially in cases with risk factors such as failure to thrive or gastrointestinal manifestation, active evaluation of ID should be encouraged.

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4P.9 The new molecular p.M177T identified in two unrelated patients with clinical features of SCO2-dependent cytochrome *c* oxidase deficiency

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Cytochrome *c* oxidase (COX, CIV) is one of the complexes of the OXPHOS system located in the inner mitochondrial membrane and catalyzing the last step of electron's transfer from cytochrome *c* to molecular oxygen. It is composed of 13 subunits encoded by mitochondrial and nuclear DNA. A correct assembly and function of COX require a substantial number of the nuclear, ancillary proteins, including SCO2, which is involved in the transport and incorporation of the cooper ions to the CuA enzymatic site on COXII subunit. Human *SCO2* gene is located on the chromosome 22q13, and contains two exons. Only the 801 bp fragment of exon 2 undergoes translation into 266 amino acid protein. Mutations in the *SCO2* gene lead to serious damage of the protein resulting in severe COX deficiency observed mainly in muscle, heart and brain. The common substitution, g.1541G>A (p.E140K) was identified at least on one allele in all so far reported patients with COX deficiency. In the group of 23 Polish patients, the common substitution was found on 84% of the studied alleles. The clinical features of the disease associated with SCO2 deficiency include early onset, fatal hypertrophic cardiomyopathy with respiratory insufficiency, encephalopathy, hypotonia and metabolic acidosis. The aim of this study was to characterize the molecular background of the disease in three patients from two unrelated families with clinically and biochemically recognized cytochrome *c* oxidase deficiency. Here we present patients with the same genotype, comprising the common mutation, g.1541G>A and a new, not described in the literature, molecular variant g.1653T>C. The new variant affects the highly conserved methionine at 177 position of the SCO2 protein (p.M177T) and was not found on 600 control alleles. Additionally, g.1653T>C substitution was predicted by SIFT BLink programme as a pathogenic mutation. Our findings indicate that the compound heterozygous genotype, p.M177T/p.E140K, is responsible for clinical manifestation of destroyed SCO2 protein.

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4P.10 Impaired mitochondrial energetic in patients harbouring *SURF1* mutations is caused by uncoupling of cytochrome *c* oxidase

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Leigh syndrome is most frequently caused by mutations of *SURF1* gene, which encodes cytochrome *c* oxidase (COX) specific assembly factor. Our previous studies suggested that fibroblasts from patients harbouring *SURF1* mutations accumulate incomplete forms of COX lacking several small nuclear-encoded subunits with decreased H^+/e stoichiometry. In experiments aimed at detailed characterization of the mitochondrial energetics, we observed 30–50% decreased respiratory capacity available for ATP synthesis (RCR_P). When using COX-specific substrates ascorbate + TMPD, the RCR_P was 7-fold lower than in controls, suggestive of deficient proton pumping of COX in patient