genes), and m.10922_15765 (MTND4–MTND6, CYB genes) with 30–42% heteroplasmy, were identified in the blood of single patients (the second mutation was confirmed by Southern hybridization). In another six children no hybridization of single MLPA probes to sequences of MTND2, MTND4, MTND6 and MTATP8 genes was found. The presence of m.3243A>G mutation was confirmed in both control cases, whereas remaining changes require verification by other methods (sequencing). No deletion was found in healthy controls. MLPA technique seems to be a useful tool in identification of heteroplasmic large-scale mtDNA deletions.

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4P.2 Determination of the pathological effect of mitochondrial DNA mutations

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It has been showed that some isolated deafness of maternal inheritance can be caused by mtDNA mutations. In collaboration with Delphine Feldmann's laboratory at the Trousseau Hospital in Paris, specialized on the study of deafness, we characterized some mtDNA mutations found in patients of this special type of deafness, in order to determine if they can be the cause of the pathology. These mutations are: C3388A, located in the coding sequence of subunit 1 of Complex I; G8078A, located in the coding sequence of subunit 2 of Complex IV; G12236A, affecting the Transfer RNA serine; and G15077A, located in the coding sequence of Cytochrome *b*, of Complex III. These mutations were found by a total sequencing of the mitochondrial genome, using a microarray technique developed by Affymetrix, Mitochip. To determine if they are responsible of the pathology, we constructed cybrids (cytoplasm hybrid). This technique consists of a cellular fusion between a cell containing mitochondrial DNA, and another cell with no mtDNA but with a known genomic DNA, which allows us to place patient's mtDNA in a known nuclear background. This way, any mitochondrial dysfunction could only be caused by patient's mtDNA. These cybrids allowed us to carry out enzymological studies. Thereby, we showed that some of these mutations caused respiratory chain dysfunctions. These results suggest that these mutations cause a cellular metabolism deficit, which implies that they could be the origin of the pathology. These studies will be completed by protein analysis, in order to study the consequences of these mutations on respiratory chain Complex assembly, as well as polarographic analysis, in order to study the global activity of the respiratory chain.

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4P.3 Characterisation of heme binding properties of *Paracoccus denitrificans* Surf1 protein

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The human *surf*1, the first gene of the *surfeit* gene locus, codes for a 30 kDa protein that is involved in cytochrome *c* oxidase (COX) assembly. Located in the inner mitochondrial membrane, Surf1 contains two transmembrane helices and a large loop facing towards the intermembrane space. Mutations in the *surf*1 gene leading to a loss of the protein are responsible for the Leigh syndrome, a fatal neurological disorder associated with severe COX deficiency [1]. In *Paracoccus denitrificans*, two Surf1 homologues were identified and named Surf1c and Surf1q for their specific role in serving a heme aa_3-type COX and a related heme ba_3-type quinol oxidase, respectively [2]. The function of Surf1 in COX biogenesis is not yet fully understood, but a role in heme *a* insertion in COX subunit I seems likely since we could recently show that it is a heme *a* binding protein [3]. To further investigate the heme binding properties of the Surf1 proteins we mutated highly conserved amino acid residues.

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4P.4 Effect of 9205delTA mutation load in the mt-*ATP6* gene on mitochondrial ATP synthase structure, function

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Missense mutations in mtDNA *ATP6* gene and replacement of several functionally important amino acids in F_O subunit *a* (F_O-*a*) represent frequent cause of mitochondrial ATP synthase dysfunction that manifest as NARP, MILS, Bilateral Striatal Lesions or other less severe syndromes [1]. A microdeletion 9205delTA represents different type of mtDNA mutation affecting the *ATP6* gene as it leads to altered splicing of *ATP8-ATP6-COX3* polycistronic transcript and results in diminished synthesis of the mRNAs for ATP synthase F_O-*a* subunit and cytochrome *c* oxidase subunit 3 (COX3). Up to now, two patients with 9205delTA mutation have been found with distinct phenotypes [2, 3] and our investigation of their fibroblasts showed different mutation loads. Therefore we have prepared transmitochondrial cybrids with varying heteroplasmy (50–100%) and studied the consequences of the mutation. We have found that the cybrid cell lines show a decrease in the synthesis of both F_O-*a* and COX3 subunits. Detailed analysis of mitochondrial ATP production, ADP-stimulated oligomycin-sensitive respiration, as well as the content of subunit F_O-*a* showed that all exert similar threshold dependence on increasing 9205delTA mutation load. A pronounced decrease in all parameters was observed when the mutation load reached about 80%. In contrast, near-linear relationship was found between the decrease in ATP production, ADP-stimulated respiration and loss of F_O-*a* subunit. The content of other F_O and F_1 ATP-synthase subunits in cybrids cell lines was normal, event at the highest mutation load. As revealed by 2D analysis of DDM-solubilised mitochondria, in near-homoplasmic 9205delTA cells we have found several incomplete forms of ATP synthase, including F_1-subunit *c* rotor subassemblies or the ATPase complex with normal mobility but lacking F_O-*a* subunit. In conclusion, our results demonstrate, that similarly as *ATP6* missense mutations, 9205delTA biochemical phenotype exhibits distinct threshold effect that originates from a gene-protein level.

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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