oxygen level fall, change their morphology, dynamics, and function, and play a major role in activating the cellular adapting mechanisms [for recent reviews see 1, 2]. Although these mechanisms are extremely important in pathophysiology, many of their aspects at the molecular level are still elusive. In addition, most studies concerning hypoxia have been carried out exposing to low oxygen levels the experimental models (animals, organs, or cells) for several hours only. Since our research group has a long experience in studying the bioenergetics of hypoxia-associated pathophysiological states, including aging [3, 4], heart and liver ischemia [5], cancer [6], and Alzheimer Disease [7], we evaluated the hypothesis that the mass, organization and function of mitochondria might be impaired when cells are exposed to prolonged hypoxia under various metabolic conditions. Therefore, as a first approach, we analyzed oxygen dependence of mitochondrial mass and function in human fibroblasts following 72 h exposition to variable oxygen pressure and energy substrates. In presence of glucose as the main fuel, the oligomycin-sensitive ATP synthesis rate of cells exposed to 1% O_2 resulted greatly decreased with respect to controls exposed to air (21% O_2) or to oxygen levels (4–6% O_2) corresponding to those present in the extracellular liquid in humans. Structural analysis of the 1% O_2 exposed fibroblasts indicated a more fragmented state and a decreased mass of the mitochondria than controls (i.e. exposed to 21% O_2); the latter was confirmed by assaying the citrate synthase activity of the cells exposed to different oxygen tensions. These results will be discussed in relation with supramolecular organization of the oxphos complexes in the mitochondrial inner membrane.

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4L.7 Cytochrome *c* oxidase biogenesis, its disorders in childhood

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Eukaryotic cytochrome *c* oxidase (CcO) is a hetero-oligomeric, heme–copper oxidase complex composed of both mitochondrially and nuclear-encoded subunits. CcO biogenesis is a complicated process that requires numerous specific assembly factors including translational activators, translocases, molecular chaperones, copper metallochaperones, heme *a* biosynthetic enzymes. We present the results of clinical, biochemical and molecular analyses in 107 CcO deficient children from our department. Methods: The activities of respiratory chain complexes were measured spectrophotometrically. The amount and protein composition were studied by BN-PAGE western blotting. DNA sequencing and PCR-RFLP were used for molecular analyses. Results: Encephalopathy was present in 90% of children, Leigh syndrome in 20%, and cardiomyopathy in 23%. Isolated CcO deficiency was found in 51 children and CcO deficiency combined with deficiency of other complexes was found in 56. In children with isolated CcO deficiency, SURF1 mutations were found in 15/51 children, SCO2 mutations in 12/51, and SCO1 mutation in one. Mutations c.845_846delCT in SURF1 and g.1541G>A in SCO2 were prevalent. At the biochemical level, SCO1, SCO2 and SURF1 deficiency resulted in tissue specific pattern of CcO assembly impairment that was not paralleled by corresponding reduction in the particular proteins' levels. Moderate to profound decrease of cellular copper was observed in muscle biopsies. MtDNA mutations were found in 7 patients with combined CcO deficiency. Conclusion: CcO deficiencies represent a heterogeneous group of diseases. Isolated CcO deficiency resulting from mutations in CcO assembly factors Surf1 and Sco2 represents the most frequently recognized causes of CcO defects in childhood. Owing to their incidence, absence of therapy and serious social–economical consequences, elucidation of the molecular mechanisms is essential for diagnostics, prevention and development of future therapeutic protocols. The reduced cellular copper levels of SCO1, SCO2 and SURF1 samples may indicate additional role of these CcO assembly proteins in copper homeostasis maintenance. The particular tissue-specific impact of SCO1, SCO2 and SURF1 deficiency suggests once again highly tissue-specific nature of respiratory chain biogenesis.

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Posters

4P.1 Detection of single large-scale mitochondrial DNA deletions by MLPA technique

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Large-scale rearrangements consist of single partial mtDNA deletions or, more rarely, partial duplications. They are heteroplasmic since they coexist with variable amounts of wild-type mtDNA. Over 150 different mtDNA deletions have been associated with known sporadic deletion syndromes: Kearns–Sayre Syndrome (KSS), progressive external ophtalmoplegia (PEO), and Pearson Syndrome (PS), although they may occasionally be identified in patients with other mitochondrial cytopathies (e.g. MELAS). The most common deletion responsible for almost 30% of deletion syndromes, contains 4977 bp and is located between nucleotides m.8469 and m.13147. Characteristic clinical features associated with large-scale mtDNA deletions include: progressive external ophtalmoplegia, generalized muscle weakness with difficulties in swallowing and articulation, short stature, deafness, conduct disturbances, delayed puberty, and endocrine dysfunction. The aim of the study was to characterize the role of the large-scale mtDNA deletions in the pathogenesis of mitochondrial disease in selected patients. Fifteen patients with mitochondrial cytopathies (including 5 KSS cases), and seven controls (5 healthy subjects, and 2 patients with known m.3243A>G mutation) were enrolled into our study. Blood samples and muscle biopsies were used as DNA source in molecular analyses. MLPA (Multiplex Ligation-dependent Probe Amplification) technique was applied in the detection of deletions. SALSA MLPA KIT P125 Mitochondria (MRC-Holland) containing 31 probes for different mtDNA sequences, and 1 mutation-specific probe for the frequent point substitution m.3243A>G (MELAS) was used. Two various deletions spanning regions: m.9169_14174 (ATP6, MTCOIII, MTND3–MTND6

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.