

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.

The study was partly supported by CMHI grant No. S111/2009.

doi:10.1016/j.bbabbio.2010.04.163

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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doi:10.1016/j.bbabbio.2010.04.164

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

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The human *surf1*, 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 *surf1* 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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doi:10.1016/j.bbabbio.2010.04.165

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*₀ subunit *a* (*F*₀-*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*₀-*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 trans-mitochondrial 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*₀-*a* and *COX3* subunits. Detailed analysis of mitochondrial ATP production, ADP-stimulated oligomycin-sensitive respiration, as well as the content of subunit *F*₀-*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*₀-*a* subunit. The content of other *F*₀ and *F*₁ ATP-synthase subunits in cybrids cell lines was normal, even 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*₀-*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.