Vondráčková et al. Large copy number variations in combination with point mutations in the *TYMP* and *SCO2* genes found in two patients with mitochondrial disorders

Supplementary Methods

Thymidine levels in plasma

Thymidine and deoxyuridine levels were analysed by reversed-phase high-performance liquid chromatography with UV detection. [1, 2]

Thymidine phosphorylase activity

Thymidin phosphorylase activity was measured spectrophotometrically in isolated lymphocytes according to Spinazzola et al.[3] Briefly, lymphocytes were isolated in Ficoll gradient. Lymphocytes were homogenized in lysis buffer (50mM Tris-HCl; pH 7.2, 1% Triton X-100; 2mM PMSF; 0.02% 2-mercaptoethanol), sonicated and centrifuged at 20000g, 4°C for 20 min. In supernatant, protein concentration was determined according to Lowry.[4] 150 mg of supernatant protein was added to reaction mixture (0.2 M Tris, pH 6.5; 0,1 M Na_2HAsO_4; 10mM thymidine) and incubated at 37°C for 30 min. The reaction was inhibited by 1 ml of 0.3M NaOH. In the reactions mixture, an amount of thymine was determined spectrophotometrically at 300 nm.

Activities of cytochrome c oxidase and citrate synthase

In patient 2, activity of cytochrome c oxidase and citrate synthase was analysed in lymphocytes isolated in Ficoll gradient, as described previously.[5]

SDS-PAGE electrophoresis and immunoblot analysis

10 μg of mitochondrial protein was separated by tricine SDS-PAGE carried out on 12% polyacrymide as described previously.[6] Proteins were electroblotted from the gels on to Immobilon™-P PVDF membranes (Millipore, Carrigtwohill, Ireland) using semi-dry transfer. The membranes were decorated with rabbit polyclonal antiserum raised against human SCO2 (1:1000), with mouse monoclonal antibodies raised against cytochrome c oxidase subunits COX1 (Abcam-Mitosciences, Eugene (OR), USA; 1 μg/ml), COX2 (Abcam-Mitosciences; 1 μg/ml) and porin (Abcam-Mitosciences; 1 μg/ml) under the same conditions as described previously.[6]

Total copper content in tissues

The total copper content in the dry matter of liver, brain and muscle tissues was assessed by FAA (Perkin Elmer 3300 AAS, Perkin-Elmer Corp., USA) or ICP-MS (Elan DRC-e Perkin Elmer SCIEX, PerkinElmer Inc., USA).

References

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