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Development of Complete Sequencing of Mitochondrial Genome as a Diagnostic Test for Patients with Suspected Mitochondrial Diseases

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Introduction. Mitochondrial diseases are a heterogeneous group of disorders that arise as a result of defects of the oxidative phosphorylation system (OXPHOS) and are, therefore, called oxidative phospholylation diseases. They are characterised with various clinical manifestations, presenting at any age with non-specific clinical symptoms and highly variable within one family. Patients with primary mitochondrial disease (caused by mutations in mtDNA or nuclear DNA-encoded mitochondrial genes) usually suffer from the multisystem organ involvement with the highest demand for the ATP – central nervous system, muscle, heart muscle, vision, liver, gastrointestinal and endocrine system. There are several baseline biochemical screening tests for mitochondrial diseases. Further diagnostics require muscle biopsy with subsequent analysis of the ATP production and enzyme activity for respiratory chain complexes. Genetic testing is performed to assess the presence or absence of mutations in mtDNA as the cause for the patient's disease.

Aim. The objective of this study is to develop a robust protocol for complete sequencing of mitochondrial genome as a diagnostic test for individuals with suspected mitochondrial diseases.

Material and Methods. Mitochondrial DNA was isolated simultaneously with genomic DNA from different suitable sources – venous or capillary blood, buccal swabs, epithelial cells from urine by appropriate extraction method (either standard phenol/chloroform, or specialised extraction kit). MtDNA was amplified as 12 overlapping fragments with length varied between 1400–1900 bp using Pfu DNA polymerase and manufacturer protocol. Ninety six synthetic oligonucleotides – four forward and four reverse primers for each fragment – were designed for the uniform direct sequencing reaction setup with single melting temperature 56°C.

Results. Fifty six mtDNA samples have been analysed using newly developed sequencing setup, and complete sequences of mitochondrial genome were obtained. Seven of them were controls from healthy persons and 49 were samples from patients with symptoms of mitochondrial disease. In two patients' samples diagnosis of mitochondrial disease (Leigh syndrome) was confirmed. The revealed mutations were mt.G13513R (p.D393N) in gene mt-ND5, and mt.9185T > C (p.L220P) in gene mt-ATP6. Additionally, unpublished mutations mtG14258A in gene ND6 was identified in patients with suspected NARP.

Conclusions. We have developed robust protocol for complete sequencing of mitochondrial genome suitable for diagnostics of mitochondrial diseases caused by mutations in the mtDNA. Application of this test will allow to avoid invasive muscle biopsy and to decrease a necessity for the hospitalization of the patients. The diagnosis of Leigh syndrome was confirmed in 2 patients.