ABSTRACT

ATP synthase represents the key enzyme of cellular energy provision and ATP synthase disorders belong to the most deleterious mitochondrial diseases affecting pediatric population. The aim of this thesis was to identify nuclear genetic defects and describe the pathogenic mechanism of altered biosynthesis of ATP synthase that leads to isolated deficiency of this enzyme manifesting as an early onset mitochondrial encephalo-cardiomyopathy. Studies in the group of 25 patients enabled identification of two new disease-causing nuclear genes responsible for ATP synthase deficiency.

The first affected gene was TMEM70 that encodes an unknown mitochondrial protein. This protein was identified as a novel assembly factor of ATP synthase, first one specific for higher eukaryotes. TMEM70 protein of 21 kDa is located in mitochondrial inner membrane and it is absent in patient tissues. TMEM70 mutation was found in 23 patients and turned to be the most frequent cause of ATP synthase deficiency. Cell culture studies also revealed that enzyme defect leads to compensatory-adaptive upregulation of respiratory chain complexes III and IV due to posttranscriptional events.

The second affected gene was ATP5E that encodes small structural epsilon subunit of ATP synthase. Replacement of conserved Tyr12 with Cys caused pronounced decrease of ATP synthase content and accumulation of hydrophobic subunit c. This phenotype was also induced by ATP5E RNAi knockdown in HEK293 cell line and indicated regulatory role of epsilon subunit in enzyme biogenesis that points to assembly and stability of F1 catalytic part and incorporation of hydrophobic c subunits into F1-c oligomer. ATP5E mutation was found only in one patient and represents the first mutation in nuclear structural gene of ATP synthase.

This thesis has been worked out in the Department of Bioenergetics, Institute of Physiology, Academy of Sciences of the Czech Republic, within collaboration with the Department of Pediatrics and Adolescent Medicine and the Institute of Inherited Metabolic Diseases, 1st Faculty of Medicine, Prague.

Key words: mitochondria, oxidative phosphorylation, ATP synthase, mitochondrial disorders, mitochondrial biogenesis, assembly factor TMEM70.