Charles University in Prague

1st Faculty of Medicine

PhD thesis summary

Mitochondrial ATP synthase deficiencies of a nuclear genetic origin

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Prague 2012

Postgraduate studies in biomedicine

Charles University in Prague and Academy of Sciences of the Czech Republic

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The full text of the thesis will be available at least five days before the PhD defense at the Department of Science and Research and International Relations of the 1st Faculty of Medicine, Charles University in Prague.

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Abstrakt

ATP syntáza je klíčový enzym buněčného metabolismu a defekty ATP syntázy patří k nejzávažnějším mitochondriálním onemocněním postihujícím dětskou populaci. Cílem této práce bylo identifikovat genetické defekty a popsat patogenní mechanismy narušení biosyntézy ATP syntázy, které vedou k izolované deficienci tohoto enzymu a projevují se jako mitochondriální encefalomyopatie s nástupem v novorozeneckém věku. Studie skupiny 25 pacientů vedla k identifikaci dvou jaderných genů zodpovědných za deficienci ATP syntázy.

První postižený gen byl *TMEM70* kódující neznámý mitochondriální protein. Tento protein byl popsán jako nový asemblační faktor ATP syntázy, první specifický pro vyšší eukaryota. Jeho velikost je 21 kDa, nachází se ve vnitřní mitochondriální membráně a není přítomný v tkáních pacientů. Mutace v *TMEM70* genu byla nalezena u 23 pacientů a ukázala se být nejčastější příčinou deficience ATP syntázy. Studie na buněčných kulturách ukázaly, že defekt enzymu vede ke kompenzačně-adaptačnímu zvýšení komplexů IV a III respiračního řetězce způsobenému posttranskripční regulací jejich biosyntézy.

Druhým postiženým genem byl *ATP5E*, který kóduje malou strukturní epsilon podjednotku ATP syntázy. Záměna konzervovaného Tyr12 za Cys způsobuje významný pokles obsahu ATP syntázy, ale zároveň akumulaci hydrofobní podjednotky c. Tento fenotyp byl také vyvolán snížením exprese *ATP5E* genu pomocí RNA interference v HEK293 buněčné linii a ukazuje na regulační roli podjednotky epsilon v biogenezi enzymu. Podjednotka epsilon pravděpodobně ovlivňuje asemblaci a stabilitu katalytické F_1 části enzymu a inkorporaci hydrofobních podjednotek c do F_1-c oligomeru. Mutace v *ATP5E* genu byla nalezena jen u jednoho pacienta a představuje první mutaci v jaderném strukturním genu ATP syntázy.

Tato dizertační práce byla vypracována na oddělení Bioenergetiky ve Fyziologickém ústavu Akademie věd České republiky, v.v.i. ve spolupráci s Klinikou dětského a dorostového lékařství a Ústavem dědičných metabolických poruch 1. lékařské fakulty Univerzity Karlovy.

Klíčová slova: mitochondrie, oxidativní fosforylace, ATP syntáza, mitochondriální onemocnění, mitochondriální biogeneze, asemblační faktor TMEM70.

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 F_1 catalytic part and incorporation of hydrophobic c subunits into F_1-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.

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

1. Introduction

1.1. Structure and function of ATP synthase

Mitochondrial ATP synthase (EC 3.6.3.14) is a nano-size rotary engine, which under oxidative conditions couples the proton gradient, generated by the respiratory chain enzymes to the synthesis of ATP from ADP and inorganic phosphate [1]. Mammalian ATP synthase has a mass of about 650 kDa and consists of matrix projecting F_1 part connected by two stalks with membrane embedded F_o part [2]. The enzyme is built of at least 16 different proteins [2], two of which, a and A6L, are encoded by mtDNA [3].

The F_1 part consists of six subunits: α, β, γ, δ, ε and inhibitor protein IF_1. Subunits γ, δ and ε [4] constitute the central stalk, while three α subunits and three β subunits form the catalytic head where ATP synthesis and hydrolysis take place. The F_o part involves 10 subunits in mammals: a, b, c, d, e, f, g, OSCP, A6L, F6, which form two domains: a peripheral stalk (or stator) and a membrane spanning part (Fig. 1).

In bovine enzyme, the c subunit is present in eight copies forming the so-called c-ring [5], which represents a rotor of the enzyme. A flow of protons, down the gradient, through the F_o part result in rotation of the c-ring rotor connected to subunits δ, ε and γ. The rotation of γ subunit causes conformational changes in the catalytic nucleotide-binding sites on the three β subunits of the F_1 part leading to ATP formation.

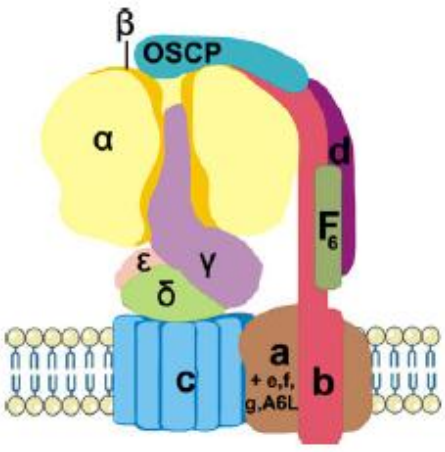

Figure 1: ATP synthase structure
Mammalian ATP synthase has a mass about 650 kDa and consists of at least 16 different subunits. It is formed by two functional domains: the catalytic F_1 part and the proton-translocating F_o part. The globular, matrix spanning F_1 part ($\alpha_3\beta_3$, IF_1) is connected by a central stalk (γ, δ, ε,) and a peripheral stalk (OSCP, d,F_6, b) to the membrane embedded F_o part (c_8, a, e, f, g and A6L). Adapted from [6].

1.2. ATP synthase biogenesis

De novo formation of ATP synthase is a stepwise process requiring the assistance of many assembly factors, and depends on the coordinated expression of nuclear and mitochondrial genomes. The assembly process of ATP synthase from individual subunits in mammals is not yet fully understood. The most valuable information regarding ATP synthase biogenesis and assembly in eukaryotes comes from studies on mutated yeasts, even though there are several differences between yeasts and mammals such as the number and location of F_o subunit c genes, ATP synthase-specific assembly factors, or factors regulating transcription of mtDNA-encoded ATP synthase genes [7, 8]. ATP synthase assembly starts with the formation of the F_1 catalytic part, which then associates with the c-ring followed by binding of the other F_o subunits (Fig. 2). It appears that the last step of monomer assembly is incorporation of two mtDNA encoded subunits, a and A6L [7, 9].

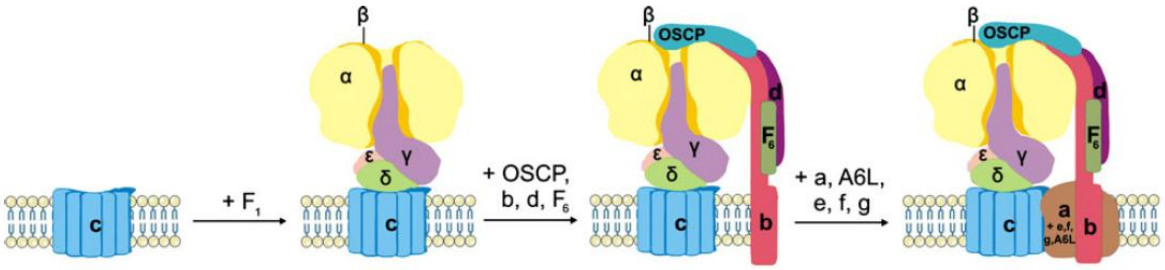

Figure 2 - The scheme of mammalian ATP synthase assembly
The current working model of mammalian F_1F_o ATP synthase step-wise formation process [6].

The biogenesis of eukaryotic ATP synthase depends on the assistance of several helper proteins that possess chaperon-type functions, specific to the assembly of ATP synthase. Additionally, there are also other factors that influence enzyme expression at different levels. This process is well described in yeast. It requires several factors involved in F_1 assembly (ATP11, ATP12, and FMC1) and F_o assembly (ATP10, ATP23). Moreover, other factors are important for mRNA stability, translation and processing of mtDNA encoded subunits (NCA1-3, NAM1, AEP1-3 ATP22, ATP25) [7, 8]. In mammals, only functional homologues of ATP11 and ATP12 have been identified [9]. It has been demonstrated that ATP11 interacts with the β subunit, and ATP12 binds selectively to the α subunit [10]. These two factors are essential for the correct assembly of functional $\alpha_3\beta_3$ oligomers. Other ancillary factors that have been found in *S. cerevisiae* are absent in higher eukaryotes and also in bacteria.

1.3. Inherited disorders of ATP synthase

ATP synthase disorders belong to a group of the most deleterious mitochondrial diseases affecting paediatric population. Two main types of ATP synthase deficiencies, which differ in pathogenic mechanism, biochemical phenotype, and clinical manifestation [7], have been described. The first group represents qualitative defects of ATP synthase caused by mutation in mtDNA encoded subunits that mostly lead to unchanged amount of non-functional enzyme. More specifically, these defects impair the F_o proton channel and thus prevent ATP synthesis. However, ATP synthase hydrolysis remains unchanged. The second group represents quantitative defects caused by mutations in the nuclear genome, which result in a selectively reduced amount of the functional enzyme. In these cases, ATP synthase content is selectively decreased to <30% of control values and the enzyme activity is profoundly reduced.

Maternally transmitted disorders of ATP synthase are caused by heteroplasmic mutations of mtDNA encoded subunit ATP6 or rarely ATP8. In contrast, disease-causing genes responsible for ATP synthase deficiency of nuclear origin remained unknown, despite increasing number of cases diagnosed.

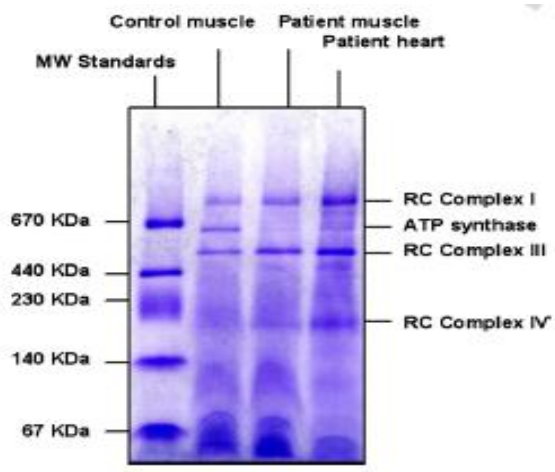

Figure 3: Reduced amount of fully assembled ATP synthase
Isolated defect of ATP synthase in muscle and heart of patient revealed by BN-PAGE [11].

In 1999, the first patient with ATP synthase deficiency of a nuclear origin was reported [12]. From that point on, the number of patients diagnosed with the same symptoms kept growing, but the affected gene was unknown [11-13]. Then in 2004, the first mutation responsible for ATP synthase disorder of a nuclear origin was reported in the gene for ATP12 assembly factor [14]. However, mutation was found only in one case and was absent in numerous other patients. In 2008, we and our collaborators succeeded to uncover a mutation in the gene for

putative mitochondrial protein TMEM70 using gene expression analysis [15] and whole genome homozygosity mapping [16]. *TMEM70* mutation was then found in most other ATP synthase deficient patients. Furthermore, within Czech-Austrian collaboration, in 2010 we also succeeded to identify the first mutation in a structural subunit of ATP synthase. One patient carried the mutation in the epsilon subunit of F_1 part of ATP synthase [17]. So far, mitochondrial disorders of ATP synthase of a nuclear origin have been shown to result from mutations in three disease-causing genes: *TMEM70* and *ATPAF2* encoding specific biogenesis factors of ATP synthase and *ATP5E* gene encoding the structural subunit epsilon of the enzyme.

2. Hypothesis and aims of the thesis

From 1999 to 2008 around 25 patients with ATP synthase deficiency presenting with the same clinical and biochemical phenotype were identified, but the genetic cause remained unknown [11-13]. The mutation in mitochondrial genome was excluded in these patients. The patients had profoundly reduced content of otherwise structurally and functionally normal enzyme. It appeared that the primary cause is a defect at the early stage of the enzyme assembly involving the formation of the F_1 catalytic part of the enzyme [11].

The aims of my thesis were to identify genetic defects and characterize pathogenic mechanism of mitochondrial encephalo-cardiomyopathies caused by isolated deficiency of ATP synthase. The thesis was based on previous studies of our department that described the first case of early-onset severe isolated defect of ATP synthase that was caused by a mutation in an unknown nuclear gene. Moreover, a unique cohort of patients with highly similar enzyme defects was diagnosed in collaboration with other research groups within several years.

Thus the specific aims of the thesis were to attempt to identify the affected gene and to learn more about molecular mechanism of these specific disorders of mitochondrial biogenesis. The thesis program represented a part of a broad, collaborative work that succeeded in identifying two new disease-causing genes. In this joint research, the effort was focused on the following points:

(i) Complementary studies with patient cell lines proving the mutation in candidate *TMEM70* gene and characterization of affected TMEM70 protein.

(ii) Testing the hypothesis that isolated defect of ATP synthase can be "compensated" at the level of biogenesis of other respiratory chain complexes.

(iii) Biochemical analysis of changes in structure, function and biogenesis of ATP synthase in patient with mutation in *ATP5E* encoding F_1 epsilon subunit.

(iv) Modeling the dysfunction of epsilon subunit by knockdown of *ATP5E* gene in HEK293 cell line.

3. Materials and Methods

This study was performed on cultured fibroblasts from patients with isolated deficiency of ATP synthase [16] and controls. All the patients showed major clinical symptoms associated with ATP synthase deficiency. In addition, for some experiments human embryonic kidney cells (HEK293) were used [18].

To identify the disease-causing gene we carried out whole-genome homozygosity mapping, gene expression analysis and DNA sequencing of patients and their healthy relatives. To prove that the candidate gene is responsible for the defects, we performed the transgenic expression of wt gene in patients' cells [16]. Import, processing and characterization of the novel protein were investigated by *in-vitro* translation followed by import into isolated mitochondria, and using HEK293 cells transfected with GFP- and Flag-tagged forms of TMEM70 as well as TMEM70 specific antibodies [19]. Moreover, model cell lines with profoundly decreased expression of *ATP5E* gene were prepared by shRNAi (Open Biosystems) in HEK293 cells [18]. The levels of *ATP5E* mRNA and 18S RNA in down-regulated cells were determinated by QT RT-PCR. To quantity the mtDNA copy number by QT RT-PCR we used 16S rRNA and D-loop as mitochondrial target and GAPDH as nuclear target [20].

The OXPHOS proteins were analyzed by one- and two-dimensional electrophoresis consisting of SDS-PAGE or/and BN-PAGE followed by Western blot analysis [16-20]. The proteins were detected using monoclonal antibodies to specific subunits of respiratory chain (MitoSciences). Quantitative detection was performed using infrared labeled secondary antibodies and Odyssey Infrared Imager (Li-Cor); the signal was quantified by AIDA 3.21 Image Analyzer software (Raytest).

To analyze mitochondrial functions [16-20] we measured respiration in digitonin permeabilized cells by Oxygraph-2k (Oroboros), $\Delta\psi_m$ by TPP^+-selective electrode, and we performed spectrophotometric analysis of respiratory chain enzymes I-IV and citrate synthase activities. Moreover, the ATP synthase hydrolytic activity sensitive to oligomycin and aurovertin was measured spectrophotometrically in ATP-regenerating system and the rate of ATP synthesis was determined by luciferase assay.

4. Results and discussion

This thesis consists of five publications. The first three are concerned with the TMEM70 protein dysfunction responsible for the most frequent isolated deficiency of ATP synthase leading to a severe mitochondrial disease. Article number one reports identification of the first mutation in the *TMEM70* gene and discovery of specific ancillary role of TMEM70 protein in biosynthesis of mitochondrial ATP synthase. The second paper presents successive biochemical and morphological characterization of mitochondrial localization and expression of the TMEM70 protein. The third paper reveals the compensatory-adaptive consequences of c.317-2A>G mutation in the *TMEM70* gene at the level of respiratory chain enzymes. Then, the other two publications deal with discovery of mutated epsilon subunit of ATP synthase as a cause of mitochondrial disease. The fourth paper reports on the first patient with a mutation in the *ATP5E* gene for epsilon subunit of the F_1 part of ATP synthase, and the fifth paper reveals the consequences of down-regulation of this subunit by RNAi in the HEK293 cell line.

1) TMEM70 mutations cause isolated ATP synthase deficiency and neonatal mitochondrial encephalocardiomyopathy

Cízková A, Stránecký V, Mayr JA, Tesarová M, Havlícková V, Paul J, Ivánek R, Kuss AW, Hansíková H, Kaplanová V, Vrbacký M, Hartmannová H, Nosková L, Honzík T, Drahota Z, Magner M, Hejzlarová K, Sperl W, Zeman J, Houstek J, Kmoch S.
Nature Genetics 2008 Nov;40(11):1288-90. Epub 2008 Oct 26.

Dysfunction of ATP synthase can be caused by mutations in mtDNA or in nuclear genes. In the time when this article was published (autumn 2008) several mutations in mtDNA encoded subunits leading to the maternally transmitted disorders of ATP synthase were known. In contrast, affected genes responsible for ATP synthase deficiency of nuclear origin were practically unknown, but the number of diagnosed patients was increasing. Only one patient had the mutation in ATP synthase assembly factor ATP12. Investigation of other cases excluded mutations in any of the 16 genes encoding enzyme subunits. However, through gene expression analysis and whole genome homozygosity mapping mutation in putative mitochondrial protein TMEM70 of about 30 kDa was uncovered. Complementation experiments revealed that ATP synthase deficiency can be rescued by the wild type *TMEM70* gene. The homozygous substitution in the splice site of the second exone (317-2A>G) resulting in aberrant splicing and loss of TMEM70 transcript was found in 23 of 25 patients and was absent in controls. One patient with a truncated TMEM70 protein was heterozygote

for this mutation and for frameshift mutation 118_119insGT. In the last patient, no *TMEM70* mutation was found. Most patients were of Roma ethnic origin and suffered from neonatal lactic acidosis, hypertrophic cardiomyopathy and/or variable CNS involvement and 3-methylglutaconic aciduria. Phylogenetic analysis showed that the TMEM70 protein is present only in multicellular eukaryotes and plants and thus represents the first ancillary factor of ATP synthase specific for higher eukaryotes.

2) Expression and processing of the TMEM70 protein

Hejzlarová K, Tesarová M, Vrbacká-Cízková A, Vrbacký M, Hartmannová H, Kaplanová V, Nosková L, Kratochvílová H, Buzková J, Havlícková V, Zeman J, Kmoch S, Houstek J. Biochimica et Biophysica Acta. 2011 Jan;1807(1):144-9. Epub 2010 Oct 16.

The purpose of the second article was to gain more information about a new factor of ATP synthase biogenesis, TMEM70. It is the third assembly-ancillary factor described in mammals in addition to ATP11 and ATP12. However, little is still known about its function and localization. Here, we report that TMEM70 is synthesized as a 29 kDa precursor, which is then imported to mitochondria and processed into a 21 kDa mature protein. The mitochondrial TMEM70 localization was proved by MS analysis, antibody detection in isolated mitochondria, and by morphological analysis of cultured fibroblasts. Moreover, fractionation of isolated mitochondria indicated that TMEM70 is part of the mitochondrial inner membrane. By 2D electrophoretic analysis (BN/SDS-PAGE) and WB we demonstrated that TMEM70 may be found in dimeric form and that the TMEM70 protein is not associated with some of the ATP synthase subunits.

Our MS analysis revealed very low cellular content of the TMEM70 protein which is in agreement with available expression profile data reporting extremely low levels of *TMEM70* transcript in human cells and tissues. Notably, *TMEM70* transcripts vary slightly among different tissues. Low cellular abundance, low transcript levels, and no tissue-specificity are characteristics for general biogenesis factors.

With a prepared anti-TMEM70 antibody we confirmed that the normal TMEM70 protein is absent in mitochondria with homozygous 317-2A>G mutation and no aberrant protein with lower molecular weight has been identified. However, a small amount of fully functional ATP synthase found in patients with a loss of *TMEM70* transcripts indicate that TMEM70 is not absolutely essential for the biogenesis of ATP synthase.

3) Compensatory upregulation of respiratory chain complexes III and IV in isolated deficiency of ATP synthase due to TMEM70 mutation

Havlícková-Karbanová V, Cízková-Vrbacká A, Hejzlarová K, Nusková H, Stránecký V, Potocká A, Kmoch S, Houstek J.
Biochimica et Biophysica Acta. Jul;1817(7):1037-43. Epub 2012 Mar 10

In this paper, we used the unique cohort group of 10 patients with the same genetic defect of homozygous 317-2A>G mutation in the *TMEM70* gene to further investigate a possible common compensatory mechanism in reaction of cells to altered mitochondrial energy provision. The dysfunction of ATP synthase leads to low ATP production, elevated mitochondrial membrane potential, and increase of ROS production. We wanted to find out how this metabolic disbalance influences the OXPHOS system. Thus, we performed a quantitative analysis of respiratory chain complexes and intramitochondrial proteases trough SDS-PAGE/WB or BN-PAGE/WB analysis. In homogenates of the patient's fibroblasts we found that the average content of ATP synthase decreased to 18% of controls amount. Conversely, the average content of respiratory enzymes III and IV increased to 133% and 163% of the control amounts, respectively. We did not find a significant change in the amount of any analyzed protease (Lon, paraplegin and prohibitins 1 and 2). The mtDNA copy number was unchanged as well. Furthermore, correlation with whole genome expression profiling determined in investigated fibroblasts did not show parallel consistent changes in the OXPHOS mRNA levels of subunits or specific assembly factors of respiratory chain complexes. The results indicate that ATP synthase deficiency leads to compensatory changes that are mainly due to posttranscriptional regulation of biogenesis of mitochondrial respiratory chain complexes.

4) Mitochondrial ATP synthase deficiency due to a mutation in the ATP5E gene for the F_1 epsilon subunit

Mayr JA, Havlícková V, Zimmermann F, Magler I, Kaplanová V, Jesina P, Pecinová A, Nusková H, Koch J, Sperl W, Houstek J.
Hum Mol Genet. 2010 Sep 1; 19(17):3430-9. Epub 2010 Jun 21.

This publication results from collaboration with Paracelsus Medical University in Salzburg.
In the group of analyzed patients with an isolated defect of ATP synthase there was one patient with a distinct clinical phenotype and no mutation in *TMEM70* gene. An Austrian girl with neonatal-onset lactic acidosis, 3-methylglutaconic aciduria and no-cardiac involvement, suffered from exercise intolerance, mild mental retardation and developed peripheral

neuropathy. As a genetic cause of the disease we identified the homozygous missense mutation A>G c.35 in second exon of the *ATP5E* gene, encoding epsilon subunit of the ATP synthase. It is the first mutation identified in the structural subunit of ATP synthase. It leads to amino acid exchange (pTyr12Cys) affecting highly conserved amino acid among eukaryotes Tyr11 located at the N-terminus which is involved in the formation of ε-δ heterodimer. Biochemically, the patient fibroblasts showed a decrease of both ATP synthase activities by 60-77% compared with the controls and an equally reduced, but fully assembled ATP synthase containing mutated epsilon. Moreover, protein content analysis revealed a decrease of either F_1 (α, β, ε) or F_o (a, d, OSCP, F6) ATP synthase subunits, except subunit c, which was found to accumulate in detergent-insoluble form. The protein content of respiratory chain complexes I, II, III and IV were normal or slightly increased in respect to the controls. Furthermore, the data from the pulse-chase experiment of metabolic labeling with ^{35}S-methionine indicates a decrease of *de novo* synthesis of ATP synthase. Thus, it seems that the isolated defect of ATP synthesis is caused by the impairment of the biogenesis of ATP synthase, which leads to a small amount of functional enzyme. The study of this patient case shows that presence of the epsilon subunit is important for proper assembly of ATP synthase.

5) Knockdown of F_1 epsilon subunit decreases mitochondrial content of ATP synthase and leads to accumulation of subunit c

Havlícková V, Kaplanová V, Nusková H, Drahota Z, Houstek J.
Biochim Biophys Acta. 2010 Jun-Jul;1797(6-7):1124-9. Epub 2009 Dec 21.

The last article completes previous publication about the importance of epsilon subunit. The epsilon subunit is the smallest and functionally less characterized subunit of the F_1 ATP synthase part, which lacks the N-terminal cleavable presequence and does not have a homolog in bacterial and chloroplast enzymes. To find out more about the role of the mammalian epsilon subunit, we down regulated expression of the *ATP5E* gene by RNAi. Silencing of the *ATP5E* gene in human HEK293 cell line leads to a decrease of activity and protein content of mitochondrial ATP synthase complex and ADP-stimulated respiration to approximately 40% of control amounts. In *ATP5E* silenced cell lines, a decreased amount of the ε subunit was accompanied by a decreased content of the F_1 subunits α and β and as well as of the F_o subunits a and d, while the content of F_o subunit c was not affected. In addition, we found the accumulated subunit c to be present in fully assembled ATP synthase complex or in subcomplexes of 200-400 kDa, which contained neither F_1 subunits α or β, nor the F_o subunits a, b or d. Thus, down-regulation of the epsilon subunit leads to a biochemical

phenotype that is very similar to the phenotype of patient with the c.35A>G mutation. Both our studies show that the ε subunit is necessary for assembly and/or stability of the F_1 catalytic part of the mammalian ATP synthase, and that it is also important for incorporation of the hydrophobic subunit c into the F_1-c oligomer during ATP synthase biogenesis.

5. Conclusions

From the results of this thesis it can be concluded that:

1)

- *TMEM70* was identified as a new disease-causing nuclear gene responsible for most cases of isolated ATP synthase deficiency. The most frequent is homozygous mutation 317-2A>G that results in aberrant splicing and loss of *TMEM70* transcript and prevents synthesis of TMEM70 protein.
- TMEM70 protein is a novel ancillary factor important for the proper assembly of ATP synthase in higher eukaryotes.
- TMEM70 is synthesized as a 29 kDa precursor, which is imported to mitochondria and processed into a 21kDa mature protein localized in the inner mitochondria membrane.
- A decreased content of ATP synthase and resulting metabolic imbalance leads to the compensatory upregulation of complex III and VI due to adaptive mechanisms originating at a posttranscriptional level, without changes in mtDNA content or the content of intramitochondrial proteases.

2)

- *ATP5E* gene was identified as the third disease-causing gene responsible for ATP synthase deficiency.
- The homozygous missense mutation in epsilon subunit (c.35A>G) was found as a cause of phenotypically rare and mild form of mitochondrial disease. It is also the first mutation in a structural subunit of ATP synthase that has been reported.
- By RNAi interference, stable HEK293 cell lines with down-regulated expression of the *ATP5E* gene were prepared.
- The cells with a c.35A>G mutation and the cell with down-regulated synthesis of the epsilon subunit manifested with similar decrease in ATP synthase complex to 30-40%. Correspondingly all F_1 and F_o subunits were reduced, except for the F_o-c subunit, which was found accumulated in a detergent-insoluble form.
- Our results showed the ε subunit is necessary for assembly and/or stability of the F_1 catalytic part of the mammalian ATP synthase, and it is also important for

incorporation of the hydrophobic subunit c into the F_1-c oligomer during ATP synthase biogenesis.

The results of this thesis contributed to better understanding of molecular genetic mechanisms responsible for inborn mitochondrial diseases caused by a dysfunction of ATP synthase and further improved our knowledge of the components and events of ATP synthase biogenesis.

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List of original articles

A) Publications in extenso related to the thesis

1. **TMEM70 mutations cause isolated ATP synthase deficiency and neonatal mitochondrial encephalocardiomyopathy.** Cízková A, Stránecký V, Mayr JA, Tesarová M, Havlícková V, Paul J, Ivánek R, Kuss AW, Hansíková H, Kaplanová V, Vrbacký M, Hartmannová H, Nosková L, Honzík T, Drahota Z, Magner M, Hejzlarová K, Sperl W, Zeman J, Houstek J, Kmoch S., Nat Genet. 2008 Nov;40(11):1288-90. Epub 2008 Oct 26
   IF = 34.284

2. **Knockdown of F1 epsilon subunit decreases mitochondrial content of ATP synthase and leads to accumulation of subunit c.** Havlícková V, Kaplanová V, Nusková H, Drahota Z, Houstek J., Biochim Biophys Acta. 2010 Jun-Jul;1797(6-7):1124-9. Epub 2009 Dec 21.
   IF = 3.688

3. **Mitochondrial ATP synthase deficiency due to a mutation in the ATP5E gene for the F1 epsilon subunit.** Mayr JA, Havlícková V, Zimmermann F, Magler I, Kaplanová V, Jesina P, Pecinová A, Nusková H, Koch J, Sperl W, Houstek J.,Hum Mol Genet. 2010 Sep 1;19(17):3430-9. Epub 2010 Jun 21.
   IF = 7.386

4. **Expression and processing of the TMEM70 protein.** Hejzlarová K, Tesařová M, Vrbacká-Cízková A, Vrbacký M, Hartmannová H, Kaplanová V, Nosková L, Kratochvílová H, Buzková J, Havlícková V, Zeman J, Kmoch S, Houstek J., Biochim Biophys Acta. 2011 Jan;1807(1):144-9. Epub 2010 Oct 16.
   IF = 5.132

5. **Compensatory upregulation of respiratory chain complexes III and IV in isolated deficiency of ATP synthase due to TMEM70 mutation.** Havlícková Karbanová V, Cížková Vrbacká A, Hejzlarová K, Nusková H, Stránecký V, Potocká A, Kmoch S, Houstek J., Biochim Biophys Acta. 2012 Jul;1817(7):1037-43. Epub 2012 Mar 10.
   IF = 4.843

B) Publications *in extenso* with different objectives

Mitochondrial DNA content and expression of genes involved in mtDNA transcription, regulation and maintenance during human fetal development. Pejznochova M, Tesarova M, Hansikova H, Magner M, Honzik T, Vinsova K, Hajkova Z, Havlickova V, Zeman J.Mitochondrion. 2010 Jun;10(4):321-9. Epub 2010 Jan 20.
IF = 3.238