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PhD thesis summary

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Study of etiopathology of mitochondrial disorders

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The full text of the PhD thesis will be available in a printed version at the Department of Science and International Relations of the First Faculty of Medicine at least five workdays before the date of the defence.

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ABBREVIATIONS

ACBD3	acyl-CoA binding domain containing 3 protein
ATAD3	ATPase family AAA domain-containing protein 3
BN-PAGE	blue native polyacrylamide gel electrophoresis
CDG	congenital disorders of glycosylation
CERT	ceramide transfer protein
CI	complex I
CII	complex II
CIII	complex III
CIV	complex IV
CoQ_{10}	coenzyme Q_{10}
CoQ_9	coenzyme Q_{10}
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
CS	citrate synthase
CV	complex V, F_1F_o-ATP synthase
DDM	n-dodecyl β-d-maltoside
DIG	digitonin
ER	endoplasmic reticulum
GCS	glucosylceramide synthase
GOLD	Golgi dynamics
HEK293	human embryonic kidney cells
IMM	inner mitochondrial membrane
KI	komplex I
KO	knock-out
LHON	Leber's hereditary optic neuropathy
MAMs	mitochondrial-associated membranes
MCSs	mitochondrial contact sites

MEGS	mitochondrial energy-generating capacity
MELAS	mitochondrial encephalomyopathy, lactic acidosis and stroke-like
MFN2	mitofusin 2
mtDNA	mitochondrial DNA
nDNA	nuclear DNA
OMM	outer mitochondrial membrane
OXPHOS	oxidative phosphorylation
P	patient
PKA	protein kinase A
PKARIα	protein kinase A regulatory inhibitor alpha
PM	plasma membrane
PMM2	phosphomannomutase 2
PPM1L	ER-resident transmembrane protein phosphatase
SCs	supercomplexes
SDH	succinate dehydrogenase
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
SM	sphingomyelin
SMS	sphingomyelin synthase
SQOR	sulfide quinone oxidoreductase
TMH	transmembrane helix
TSPO	translocator protein
WES	whole exome sequencing
σ-1 receptor	Sigma-1 receptor

ABSTRAKT

Mitochondriální onemocnění představují klinicky, biochemicky i geneticky heterogenní skupinu dědičných onemocnění, jež prevalence je přibližně 1:5 000 živě narozených dětí. Společným znakem těchto onemocnění je narušení mitochondriálního energetického metabolismu. V současné době je známo více než 400 genů asociovaných s mitochondriálním onemocněním, avšak 45 % pacientů s podezřením na mitochondriální onemocnění je stále bez potvrzené genetické příčiny. Pomocí sekvenování nové generace nacházíme nové kandidátní geny anebo varianty, které by mohly stát za příčinou onemocnění. Abychom mohli potvrdit kauzalitu těchto nově nalezených genů a variant, je třeba charakterizovat deficit pomocí řady biochemických metod.

Cílem této práce bylo studovat funkci proteinu ACBD3 na úrovni mitochondriálního energetického metabolismu v ne-steroidních buňkách HEK293 a HeLa a potvrdit tak kauzalitu genu *ACBD3* u pacientky s kombinovaným deficitem systému oxidativní fosforylace (OXPHOS). Druhým cílem bylo potvrdit kauzalitu dvou nových variant v genech *MT-ND1* a *MT-ND5*, kódujících strukturní podjednotky komplexu I (KI) dýchacího řetězce. Třetím cílem práce bylo studovat tvorbu superkomplexů u pacientů se vzácnými dědičnými metabolickými poruchami.

V předkládané dizertační práci se podařilo pomocí funkční studie proteinu ACBD3 prokázat, že tento protein nemá významnou funkci v mitochondriích, nicméně je nezbytný pro udržení struktury Golgi. Dále se podařilo potvrdit kauzalitu dvou nových variant v genech *MT-ND1* a *MT-ND5* a byla vytvořena hypotéza dopadu mutace v genu *MT-ND1* na mechanismus funkce KI. Též byla charakterizována kohorta pacientů z České a Slovenské republiky s deficitem KI, způsobeným mutacemi v mitochondriálně kódovaných strukturních podjednotkách KI. V neposlední řadě bylo ukázáno, že u pacientů s dědičnými poruchami glykosylace dochází k zvýšené tvorbě superkomplexů.

Klíčová slova: mitochondrie, mitochondriální onemocnění, ACBD3, *MT-ND1*, *MT-ND5*, superkomplexy

ABSTRACT

Mitochondrial disorders are a clinically, biochemically and genetically heterogeneous group of inherited disorders with a prevalence of about 1:5 000 live births. A common sign of those disorders is disruption of mitochondrial energetic metabolism. To this day, more than 400 genes have been associated with mitochondrial disorders, but 45% of patients are still without a genetic diagnosis. Using next-generation sequencing, new candidate genes or variants are found. To confirm the causality of those newly found genes or variants, biochemical characterisation using a plethora of various methods is necessary.

The first aim of this thesis was to study the function of ACBD3 protein on mitochondrial energetic metabolism in non-steroidogenic cells HEK293 and HeLa and to confirm the causality of the *ACBD3* gene in a patient with combined oxidative phosphorylation (OXPHOS) deficit. The second aim was to confirm the causality of two novel variants in *MT-ND1* and *MT-ND5* genes, which encode structural subunits of complex I (CI) of the respiratory chain. The third aim of the thesis was to study the formation of supercomplexes (SCs) in patients with rare metabolic diseases.

Using functional studies, we showed in this thesis that ACBD3 protein has no essential function in mitochondria but plays an important role in the maintenance of Golgi structure. Moreover, the causality of two novel variants in *MT-ND1* and *MT-ND5* genes was successfully confirmed and a hypothesis about the impact of the mutation in the *MT-ND1* gene on the mechanism of CI function was formulated. Furthermore, a cohort of Czech and Slovak patients with CI deficit caused by mutations in mitochondrial DNA-encoded structural subunits of CI was characterised. Last but not least, it was shown that in patients with congenital disorders of glycosylation the formation of SCs is increased.

Keywords: mitochondria, mitochondrial disorders, ACBD3, *MT-ND1*, *MT-ND5*, supercomplexes

1 INTRODUCTION

Mitochondria are dynamic organelles with many functions. The hallmark of mitochondria is cellular energy generation via oxidative phosphorylation (OXPHOS). Mitochondria contain their DNA (mtDNA), altogether mtDNA encodes 37 genes (13 OXPHOS proteins, 22tRNA and two rRNA). The approx. 1500 remaining proteins of the mitochondrial proteome are encoded in the nuclear genome and transferred to the mitochondria via a sophisticated import system [1]. Unlike the mutation in nuclear DNA (nDNA) where standard Mendelian inheritance occurs, the mtDNA mutations are maternally inherited. Moreover, due to multiple copies of mtDNA within a cell, these copies could all be identical in sequence and this condition is called homoplasmy. But, as a result of replication errors, inefficient DNA repair, oxidative stress, or inheritance of mutated copies, a percentage of copies could carry a mutation (heteroplasmy).

1.1 Oxidative phosphorylation

The majority of ATP in a cell is generated by the OXPHOS system, which is conserved from bacteria to higher eukaryotes. The OXPHOS comprises five multiprotein enzymes (Complex I to V; CI to CV) and two mobile electron carriers (coenzyme Q_{10} (CoQ_{10}) and cytochrome *c*. The OXPHOS system pumps protons across the inner mitochondrial membrane (IMM) generating a proton gradient and this proton-motive force is used by F_1F_o-ATP synthase (CV) to phosphorylate ADP to ATP. The OXPHOS complexes are not static entities in the IMM, they are dynamic and are aggregated in different stoichiometric combinations to form supercomplexes (SCs). Currently, the most accepted model is the so-called “plasticity model” or “dynamic aggregate” where both organizations – SCs and free OXPHOS complexes coexist. This model proposes dynamic changes in the complexes between the “free” state and SCs formation in response to varying energetic demands and it is assumed that the isolated complexes are the preassembly state before their association into SCs [2–4].

1.2 Mitochondrial disorders

Mitochondrial disorders are OXPHOS dysfunction or other defects of mitochondrial structure and function. Their minimum birth prevalence is 1:5000 [5,6] and more than 400 genes have been reported to be associated with mitochondrial disorders [7]. These genes are not only for OXPHOS proteins and their assembly factors, but also genes for mtDNA maintenance, mitochondrial gene expression, quality control, import, and dynamics or other processes,

including the tricarboxylic acid cycle or pyruvate metabolism. Mitochondrial disorders may be inherited by many different genetic mechanisms: maternal in the case of mtDNA mutations and autosomal recessive, dominant and X-linked in the case of nuclear gene mutations. Mitochondrial diseases have a broad range of phenotypic and biochemical presentations which makes it challenging to diagnose them. Previously, the traditional diagnostic approach ("biopsy first") of the evaluation of the patient body fluids together with the analysis of the OXPHOS enzymes activities in muscle tissue, followed by Sanger sequencing of single candidate genes ("from function to gene") was used. But, in the last years, next-generation sequencing (e.g. whole exome sequencing (WES) and whole genome sequencing) has become the first-line routine technology. Thus, invasive muscle biopsy is performed less often but still is indispensable in some cases [8]. Functional studies enabled diagnostic uplift from 36% to 55% [7]. For the characterisation of the candidate gene/variant, an invasive tissue biopsy (muscle or at least skin biopsy) followed by functional analysis must be performed.

1.3 The importance of mitochondrial cholesterol

Cholesterol is transported to the mitochondria through the lipid transfer proteins at the mitochondrial contact sites (MCSs) or by cytosolic, diffusible lipid transfer proteins. Although, the amount of cholesterol in the mitochondria is approx. 40 times lower compared to the plasma membrane (PM) [9], mitochondrial cholesterol is an important precursor for steroids, oxysterols, and hepatic bile acid and is also an essential part of the mitochondrial membranes. Disruption of mitochondrial cholesterol content has been described in a wide range of pathophysiological conditions [10–16]. Mitochondrial cholesterol plays an important role in mtDNA maintenance and gene expression. Nucleoprotein structures containing mtDNA (nucleoids) are linked with membrane-associated replication platforms which are abundant in cholesterol [17]. Disruption of cholesterol homeostasis, e.g. by altered expression of the ATAD3 (ATPase family AAA domain-containing protein 3) gene, impairs mtDNA topology and mitochondrial protein synthesis [17–21].

1.4 Mitochondrial cholesterol import

Cholesterol import to the mitochondria has been studied over decades, predominantly in the context of steroidogenesis [16]. The major route for cholesterol import to the mitochondria is from the endoplasmic reticulum (ER) and lysosomes through multiple mitochondrial MCSs. The ER-mitochondria contact sites (well-known as mitochondrial-associated membranes (MAMs)) are implicated in the transport of phospholipids and cholesterol [22]. The

mitofusin 2 (MFN2) protein and Sigma-1 receptor (σ-1 receptor) play important functions in cholesterol import into mitochondria. The MFN2 is mitochondrial GTPase tethering the ER with mitochondria and its depletion leads to decreased synthesis of progesterone [23]. Similarly, the knockdown of the σ-1 receptor, another protein tethering MAMs with function in multiple signalling pathways, reduces progesterone synthesis by 95% [24]. Nevertheless, the mechanism of cholesterol transport into mitochondria remains poorly understood [16,25–27].

1.4.1 The STARD1 (StAR) pathway of cholesterol transport into mitochondria

Transport of cholesterol from lipid droplets and from ER to the outer mitochondrial membrane (OMM) mediates the STARD1. The STARD1 is rapidly synthesized in response to hormonal stimulation and mutations in the *STARD1* gene are associated with congenital adrenal hyperplasia [28]. After hormonal stimulation, STARD1 is translocated to the OMM where it is fully activated by PKA (protein kinase A). But the mechanism of STARD1-mediated cholesterol import is not yet exactly clear [16]. Several proteins have been described as interacting partners of STARD1, forming a large complex for cholesterol transport across mitochondrial membranes but the exact composition and mechanisms are still debated. A multiprotein complex (named transduceosome), consisting of the STARD1, VDAC1, TSPO, ACBD3, PKARIα (type I PKA), ATAD3 and CYP11A1 (cytochrome P450), with a function in transporting cholesterol into mitochondria was described by a research group led by professor V. Papadopoulos [29,30]. ACBD3 (acyl-CoA binding domain containing 3 protein) is an A-kinase anchoring protein for PKARIα (protein kinase A regulatory inhibitor alpha), so the transducesome would bring together components for activation of STARD1 and positions STARD1 close to the contact sites [29,31]. Close contact between OMM and IMM is mediated by the ATAD3 protein [32,33]. The TSPO (translocator protein) contains a cholesterol recognition amino acid consensus motif, mediates cholesterol binding and oligomerizes to form a cholesterol-transporting channel upon hormone stimulation [34–39]. But as was mentioned above, mitochondrial cholesterol import has been studied particularly in steroidogenic cells and not much research has been done in non-steroidogenic cells.

1.5 The ACBD3 protein

Humans express seven highly conserved Acyl-CoA-binding proteins (ACBD1–ACBD7). A common feature of this protein family is the ACB domain, responsible for the binding of long-chain fatty Acyl-CoA esters. ACBD3 is the largest protein of this family, consisting, apart from the ACB domain, of a coiled-coil domain in the middle and a Golgi dynamics

(GOLD) domain on the C terminus. The GOLD domain is responsible for multiple protein interactions and may be used to stabilize peripheral membrane proteins at intracellular membranes [40–42]. A high expression level of ACBD3 protein (The Human Protein Atlas[1]; [43]) was found in some organs of the digestive system, brain, prostate, placenta, and bone marrow; medium expression is characteristic for male and female reproductive tissues. The antibody validation profile (The Human Protein Atlas[1]) localised ACBD3 in Golgi and is a membrane-bound or membrane-associated protein. Inferring from sequence similarity, ACBD3 is probably also localized in mitochondria [43–45]. The MitoCarta predictions[2] [46], mark ACBD3 as "possible mito". According to published research, ACBD3 is localized in the ER, Golgi, mitochondria, PM, and cytosol [29,47–52]. The ACBD3 protein plays various roles in the cell: it is a Golgi-ER tether [51] or Golgi scaffold protein [47,49], it has a function in vesicle trafficking (sphingolipid transport) [50], the import of cholesterol into mitochondria/steroid synthesis [29,53], and the regulation of cellular iron uptake [54,55]. Furthermore, the ACBD3 protein participates in the replication of multiple members of the picornavirus family [56–59]. Multiple ACBD3-protein interactions are visualised in Figure 1.

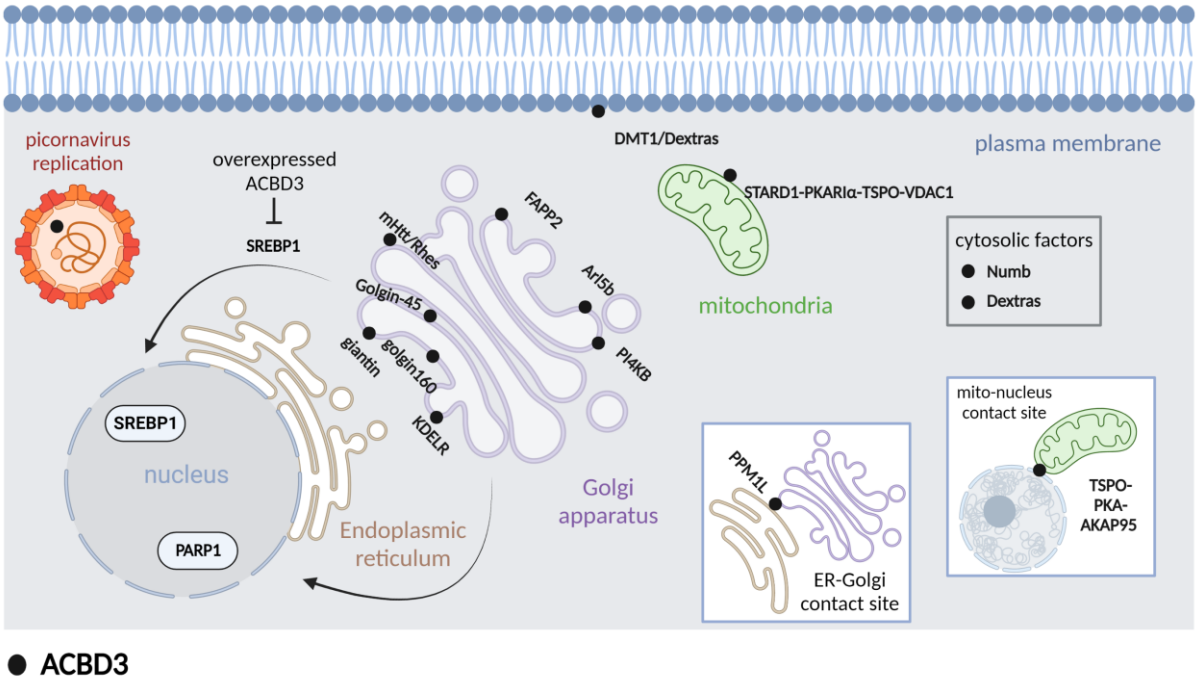

Figure 1: Schematic picture of diverse roles of ACBD3 and its interaction partners in various cellular compartments. "Created with BioRender.com."

[1] accessed on July 25th 2022 at https://www.proteinatlas.org/
[2] MitoCarta 3.0; accessed on July 25th 2022 at https://www.broadinstitute.org/mitocarta

2 AIMS OF THE THESIS

The next-generation sequencing is the first-line approach in the diagnosis of patients with suspected mitochondrial disorders. The use of WES and whole genome sequencing leads to the discovery of new candidate genes or new variants in known disease genes. To evaluate the role of the candidate gene/variant in the disease, extensive functional analyses are often necessary.

The aims of the thesis were the functional characterisations of the candidate genes and variants to confirm their pathogenicity. Firstly, in a patient with suspected mitochondrial disorders, a rare variant in the *ACBD3* gene was found by WES. Up to date, the *ACBD3* gene has not been found to be associated with disease in men. To assess its possible pathogenicity, we decided to study the function of the ACBD3 protein in mitochondria using the ACBD3-knockout (KO) cell lines. Secondly, using mtDNA sequencing, two novel mutations in the *MT-ND1* and *MT-ND5* genes were found. To confirm the pathogenicity of those variants, functional analyses in muscle and fibroblasts from the patients were necessary. Thirdly, the study of mitochondrial SCs helped us to further characterise other rare metabolic diseases.

The specific aims of the thesis were:

A. Study of the role of ACBD3 protein in mitochondria and Golgi in HEK293 and HeLa cells
B. Description of the impact of novel variants in mtDNA-encoded genes on mitochondrial energetic metabolism
C. Study of mitochondrial SCs in a patient with rare metabolic disorders

3 MATERIAL AND METHODS

3.1 Materials

3.1.1 Cell Cultures

Human embryonic kidney cells (HEK293, ATCC® CRL-1573™), HeLa (ATCC® CCL-2™) and control skin fibroblasts (ATCC® PCS-201-010™ and Lonza CC-2509) were purchased from the American Type Culture Collection (Rockville, Maryland, USA) or Lonza (Basel, Switzerland). Fibroblasts from patients and healthy controls were derived from skin biopsies after informed consent.

Cells were cultivated as described previously [60].

3.1.2 Muscle tissue

Muscle biopsies (*m. tibialis anterior* (P1, P3, P7, and control for P8); *m. triceps surae* (P4, P5, P6, P8, P9, P10, P11, and P14)) were obtained after informed consent. Control for P14 is skeletal muscle from adult control obtained during orthopaedic surgery.

3.2 Methods

3.2.1 Preparation of HEK293 and HeLa ACBD3-KO cell lines

ACBD3-KO was introduced into HEK293 and HeLa cells by the CRISPR/CAS9 system (Clustered Regularly Interspaced Short Palindromic Repeats) as described previously [60].

3.2.2 Isolation of mitochondria

Isolations of mitochondria from cells and muscle were performed as described previously [60] and [61], respectively.

3.2.3 Electrophoresis and WB

The steady-state level of OXPHOS protein complexes was studied by blue native polyacrylamide gel electrophoresis (BN-PAGE) and the steady-state level of OXPHOS protein subunits was studied by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), as described previously [60]. To study the steady-state level of SCs, solubilisation with digitonin (DIG) was performed at a final ratio of 7 mg DIG/mg protein in a buffer containing 1.5mM Aminocaproic acid; 0.05M Bis-Tris; 2mM EDTA (pH = 7.0) at 4°C for 15 min. After DIG solubilisation, samples were centrifuged at 20,000g, 4°C for 20 min

and supernatants were used for downstream analysis. 8–15 µg of protein (determined by BCA assay, (Thermo Fisher Scientific) was loaded per lane and separated by NativePAGE™ 3–12% Bis-Tris Mini Protein gels (Thermo Fisher Scientific). To analyse the steady-state level of VDAC1 protein from n-dodecyl β-d-maltoside (DDM)-solubilized mitochondria, 8 µg of protein were lysed in RIPA buffer and denatured for 30 min at 37°C in SDS-sample buffer. Samples were then separated by tricine SDS-PAGE using 12% (w/v) polyacrylamide mini gels (MiniProtean® 3 System; Bio-Rad).

3.2.4 High-Resolution Respirometry

High-Resolution Respirometry was performed as described previously [60].

3.2.5 Analysis of mtDNA content

Analysis of mtDNA content was performed as described previously [60].

3.2.6 Flow cytometry measurement of dihydroethidium-stained cells

Analysis of ROS production was performed as described previously [60].

3.2.7 Confocal and transmission electron microscopy

Microscopic analyses were performed as described previously [60].

3.2.8 Lipidomics[3]

Lipidomics analysis was performed as described previously [60].

3.2.9 Measurement of sphingomyelin synthase activity

Analysis of sphingomyelin synthase (SMS) activity was performed as described previously [60].

3.2.10 Measurement of OXPHOS enzyme activities

The activities of respiratory chain complexes[4] were measured as described previously [61].

[3] This analysis was performed at the Laboratory of Metabolism of Bioactive Lipids, Institute of Physiology, Academy of Science, Czech Republic

[4] Measurement was performed as a part of rutine diagnostics in Laboratory for study of mitochondrial disorders

3.2.11 Computational structural analyses[5]

The visualisations of respiratory complexes and their components were rendered by PyMol software, using atomic coordinates of human CI (PDB ID: 5XTD) and coordinates of active and inactive forms of mouse CI (PDB ID: 6G2J and 6G72, respectively) [62] The effect of mutations on protein structure and stability was predicted using DynaMut software [63].

Multiple sequence alignment was performed using the ConSurf server [64]. The resulting alignment contains 2000 unique sequences that in equal intervals sampled the representative homologous sequences, sharing identity between 50 and 95% with the human ND1.

[5] Computational structural analyses was performed in collaboration with Assoc. Prof. Václav Martínek Ph.D., Department of Biochemistry, Faculty of Science, Charles University, Prague, Czech Republic.

4 RESULTS AND DISCUSSION

4.1 Results and discussion related to the aim A) The role of ACBD3 protein in mitochondria and Golgi in HEK293 and HeLa cells

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4.1.1 The role of ACBD3 protein on mitochondrial metabolism

To study the role of ACBD3 protein on mitochondrial metabolism, three HEK293 and one HeLa ACBD3-KO clones were prepared. Using a wide range of analyses (representation of OXPHOS protein complexes and subunits, mitochondrial respiration, ROS production, mitochondrial ultrastructure, mtDNA relative quantification, and lipidomics analysis) in ACBD3-KO cells, it was shown that the ACBD3 protein is dispensable for the proper function of the OXPHOS and its absence has no notable effect on the level of cholesterol (both mitochondrial and cellular). To our knowledge, this study is the first research focused on the role of ACBD3 in mitochondrial functions. It is supposed that there is an alternative pathway of cholesterol transport into mitochondria. Along with ACBD3, ACBD1 and ACBD2 are also mitochondrial proteins [46]. ACBD1 was discussed previously as a part of the multiprotein complex transporting cholesterol into mitochondria [65–68], but unlike ACBD3, its role was not described in detail. An ACBD1-dependent formation of mitochondrial pregnenolone was described in C6-2B glioma cells [69] and the depletion of ACBD1 in MA-10 and R2C Leydig cells caused reduced human chorion gonadotropin-stimulated steroidogenesis and decreased progesterone production, respectively [70,71]. Equivalently to ACBD3, ACBD1 also binds TSPO at the OMM and IMM contact sites and stimulates the transport of cholesterol into mitochondria. In mitochondria, ACBD1 directly promotes the loading of cholesterol on the CYP11A1 enzyme [72]. Similarly, ACBD2 protein might participate in cholesterol transport into mitochondria. The ectopic expression of the ACBD2 isoform A led to increased basal and hormone-stimulated steroid formation in MA-10 Leydig cells [73]. Albeit most of the research focusing on cholesterol transport into mitochondria has been carried out in the context of steroidogenesis, a new mechanism of cholesterol transport into mitochondria in non-steroidogenic cells has been described [25]. Recently, a new protein GRAMD1B together with the Arf1 GTPase were described as proteins indispensable for cholesterol transport from ER to mitochondria in the C2C12 mouse

myoblast cell line. Their depletion led to a significant decrease in mitochondrial cholesterol content, resulting in mitochondrial dysfunction [25].

4.1.2 The role of ACBD3 protein in Golgi

Due to the primary localisation of ACBD3 in Golgi, the structure of this organelle was examined. In ACBD3-KO cells, Golgi was extremely fragmented and disorganized and no characteristic ribbon-like structure and stacked cisternae were observed. But the significantly altered Golgi structure did not affect the glycosylation pattern of the LAMP2 glycoprotein nor the level of selected Golgi proteins involved in the maintenance of Golgi structure (GM130, GRASP65 and GRASP55). Those results confirm previously published data on an ACBD3-downregulated HeLa cell line [50]. Altogether, the data demonstrate the indispensability of the ACBD3 protein in Golgi stacking. Contrary to a double KO of GRASP55 and GRASP65 [74], the disruption of the Golgi structure in ACBD3-KO cells did not affect the level of Golgi proteins (GRASP55, GRASP65, and GM130), participating in the assembly of the apparatus, nor the glycosylation pattern of the LAMP2 glycoprotein and the level of hexosylceramides. Albeit we demonstrated that ACBD3-deprivation mediated defect of Golgi maintenance did not affect the glycosylation pattern of LAMP2 glycoprotein, another study recently described a disruption of the glycosphingolipid metabolism in an ACBD3-downregulated HeLa cell line [50]. Thus, it remains obscure whether and to what extent the absence of ACBD3 affects the functions of the Golgi.

An interesting founding was the significantly diminished level of coenzyme Q_9 (CoQ_9), but normal CoQ_{10} in both whole cells and mitochondria in ACBD3-KO cells. In humans, CoQ_9 and CoQ_{10} are synthesized by the same PDSS1/2 heterotetramer [75] in the first step of the mitochondrial part of the CoQ biosynthesis. The function of human CoQ_9 and the regulation mechanism specifying if CoQ_9 or CoQ_{10} will be synthesized remains unclear. From our results, it seems that ACBD3 might somehow assist in the regulation of the specificity of PDSS1/2 for chain length formation. The BioPlex 2.0 study of protein-protein interactions identified the ACAD9 protein as a PDSS1 and PDSS2 interacting partner [76], but the role of ACAD9 in CoQ biosynthesis is not yet known. ACAD9 is also an Acyl-CoA binding protein and seems to have a similar role in the replication of some picornaviruses as ACBD3 [77]. Hypothetically, ACAD9 and ACBD3 could have similar but yet unknown functions in the regulation of PDSS1/2. As was already mentioned above, both CoQ biosynthesis and the function of CoQ_9 in humans still require much research to be carried out.

Moreover, decreased level of sphingomyelins (SMs), but a normal level of ceramides and hexosylceramides in both, ACBD3-KO cells and mitochondria, was found. But the *in situ* activities of SMS and glucosylceramide synthase (GCS) remain comparable with controls. Altogether, this suggests that ceramide is not effectively transported to the Golgi as a substrate for SMS. Transport of ceramides from ER to the Golgi for the synthesis of SM is CERT (ceramide transfer protein)-dependent, but transport of ceramides for GlcCer synthesis CERT-independent or CERT does not play a major role [78,79]. We hypothesize that decreased level of SM, together with a normal SMS activity in ACBD3-KO cells, could be caused by impaired transport of ceramides from ER to Golgi. This is in accordance with previously published data [51], indicating for the first time the role of ACBD3 in the recruitment of PPM1L (ER-resident transmembrane protein phosphatase) to the ER-Golgi MCSs, which seems to be indispensable for the activation of CERT. We supposed that the ACBD3 protein is fundamental in the activation of CERT via PPM1L. The mechanism of delivery of ceramides from ER to the site of GlcCer synthesis remains unknown [78], but according to our results, the transport is probably ACBD3-independent. Recently, increased SM and GlcCer levels were observed in an ACBD3-downregulated HeLa cell line [50]. This distinction could be related to the amount of ACBD3 residual protein in the downregulated HeLa cell line, as discussed previously [80,81].

4.2 Results and discussion related to the aim B) Description of the impact of novel variants in mtDNA-encoded genes on mitochondrial energetic metabolism

Tereza Danhelovska, Hana Kolarova, Jiri Zeman, Hana Hansikova, Manuela Vaneckova, Lukas Lambert, Vendula Kucerova-Vidrova, Kamila Berankova, Tomas Honzik, and Marketa Tesarova. "Multisystem Mitochondrial Diseases Due to Mutations in MtDNA-Encoded Subunits of Complex I." BMC Pediatrics 20, no. 1 (January 29, 2020): 41. https://doi.org/10.1186/s12887-020-1912-x. (IF = 2.765)

Rákosníková Tereza, Kelifová Silvie, Štufková Hana, Lišková Petra, Kousal Bohdan, Martínek Václav, Honzík Tomáš, Hansíková Hana, Tesařová Markéta. "A rare variant m.4135T>C in the *MT-ND1* gene leads to LHON and altered OXPHOS supercomplexes." (manuscript prepared for submission)

4.2.1 Characterisation of mitochondrial disorders caused by a mutation in *MT-ND* genes including two novel variants

Mitochondrial disorders caused by mtDNA mutation in genes for structural subunits of CI were diagnosed in 15 patients (P1–P15) from 14 families. Altogether, 10 different heteroplasmic mtDNA mutations in the *MT-ND1*, *MT-ND3*, *MT-ND5* and *MT-ND6* genes were found, including two novel variants: m.4135T>C (p.Tyr277His) in *MT-ND1* (P14) and m.13091T>C (p.Met252Thr) in *MT-ND5* (P8). Similarly to other reports [82–84], the most frequent mutation in the *MT-ND5* gene in our group of patients was m.13513G>A. Most of our patients manifested during childhood or adolescence, with Leigh or mitochondrial

encephalomyopathy, lactic acidosis and stroke-like (MELAS) syndrome. Less frequent was the onset of optic neuropathy followed by multisystem symptoms resulting in Leber's hereditary optic neuropathy (LHON)/MELAS overlap syndrome.

4.2.1.1 Clinical symptoms in patients with novel variants

Patient 14 is a 40 years old man who was asymptomatic until the age of 37 years when decreasing visual acuity bilaterally occurring only during physical exercise started. Patient 8 is a 35 years old woman whose first symptoms appeared at the age of 10 years when a frequent attack of migraine started. At the age of 26, she developed myopathy, optic neuropathy and secondary epilepsy. This is compatible with our observation that any symptom from the broad phenotypic spectrum of MELAS syndrome may come first and stay isolated for a long period [85].

4.2.1.2 Characterisation of patients with MT-ND genes mutation by several biochemical methods

In isolated muscle mitochondria, the activity of CI normalized to the activity of citrate synthase (CI/CS) was decreased or borderline low in 9/11 analysed patients (82%), and the activity of the respiratory chain CI+III/CS was reduced in 10/11 patients (91%). Compensatory increased activities of CII+III/CS and CIII/CS were found in 4/11 patients (36%), while CIV/CS activity was decreased in 4/11 patients (36%). No significant correlation between enzymatic activities and the heteroplasmy of mtDNA mutations was observed. We showed, that CI+III activity in muscle normalized to the activity of CS (serving as a control enzyme) is a good biochemical indicator for CI deficiency caused by a mutation in *MT-ND* genes. This fact may be due to the CI assay measuring only the redox activity of the enzyme, which takes place within the peripheral arm, while mutations in the membrane arm subunits may theoretically result in ostensibly normal enzymatic activity.

4.2.1.3 Functional characterisation of the novel variants m.4135T>C in MT-ND1 and m.13091T>C in MT-ND5 genes

In the case of novel variants, analyses of muscle biopsies were necessary to confirm the pathogenicity of those variants. Histochemistry in the skeletal muscle biopsy revealed focal subsarcolemmal accumulation of the SDH (succinate dehydrogenase) reaction product in approx. 5% and 3% of muscle fibres in P14 and P8, respectively. The activity of CI was decreased to 33% of the lower limit of the reference range in the muscle of P14, while P8 remain at the lower limit of the control. The activity of CI+III was decreased to 54% and

38%, respectively. The activities of CII+III and CIII were increased in both patients (probably as the compensatory impact of CI deficiency).

Analysis of DDM-solubilised mitochondria from skeletal muscle reveals the decreased level of CI holoenzyme to approx. 74% in P8, while in P14 only a slightly decreased level of CI (approx. 80%) was found. Due to the ambiguous results from spectrophotometry and BN-PAGE in muscle tissue from P14, a more detailed analysis of OXPHOS complexes in mitochondria from available tissue, cultured skin fibroblasts, was performed. While in DDM-solubilized mitochondria, only a minimal reduction of the CI level and no accumulations of CI assembly intermediates were found, analysis of DIG-solubilised mitochondria reveals a decreased level of CI-containing SCs and increased CIII-dimer in fibroblasts. Due to the limited amount of obtained tissue, analysis of SCs from muscle was not performed.

The amount of other OXPHOS complexes remains unchanged in the P8 muscle except F_1F_o-ATP synthase, where a mild increased amount of free F_1 part was observed. In P14, a slightly decreased level of CIV and a mildly elevated level of CV without accumulation of CV subcomplexes were found.

The increased level of CIII-dimer, together with elevated CII, CII+III, CIII and CS enzyme activities were also reported in 143B cybrids carrying a homoplasmic m.3571dupC (p.Leu89fsPro*13) in *MT-ND1* gene [86] or in L929dt mouse fibroblasts carrying two homoplasmic mutations in *MT-ND2* gene [87]. While CIII-dimer remains stable in case of decreased formation of CI-containing SC, the steady-state level of CIV was reduced, probably due to diminished stability in the absence of SCs formation [86], similar founding was observed in fibroblasts from P14. Recently, a novel m.3955G>A variant in the *MT-ND1* gene was found in two patients with Leigh syndrome [88]. Analysis of cybrids cells with mutation loads of 87% and 98%, respectively, showed a decreased level of ND1 protein subunit and a significantly reduced level of mature CI. CI-containing SCs were significantly decreased in both cybrids cell lines when detected by NDUFS2, but in UQCRC2 or COXIV detections, similar signals across WT and mutant cybrids cell lines were found [88]. The activity of CI was significantly reduced, but CII, CII+III, CIII, and CIV activities remain comparable to controls [88].

While in the case of P8, the pathogenicity of the variant m.13091T>C in the *MT-ND5* gene was confirmed by spectrophotometric measurement of OXPHOS enzyme activities and BN-PAGE/WB analysis from the patient's muscle, in the case of P14 the results from BN-PAGE were not entirely convincing. Due to that, we focused more deeply on the position of the

mutation in the CI structure. The affected ND1 subunit is localised in the membrane part of the CI close to the matrix part of the enzyme. Human ND1 has eight transmembrane helices (TMH) and Tyr277 is located at the matrix end of TMH7. Tyr277 is highly conserved across mammals, but in a larger group of organisms (including prokaryotes), position 277 is conserved for hydrophobic residues. Using the MitImpact [89], 11 out of 16 pathogenicity predictors evaluate the m.4135T>C variant as pathogenic.

To predict the effect of the missense variant m.4135T>C (p.Tyr277His) in the *MT-ND1* gene on the structure and function of CI, DynaMut software was used [63]. DynaMut integrates their graph-based signatures along with normal mode dynamics to generate a consensus prediction of the impact of a variant on protein stability, thus allowing prediction of both stabilizing and destabilizing effects of the missense variant on the protein. Tyr277His mutation is predicted to be destabilizing using the cryo-electron microscopy structure of the mouse mitochondrial CI in the active state (PDB:6G2J) [90]. Interestingly the mouse mitochondrial CI in the inactive state (PDB:6G72) is predicted to be slightly stabilized by the Tyr277His mutation. The inactive form stabilisation effect of the mutation could be explained by forming the new inter-subunit H-bond between His277 (subunit ND1) with Asn232 of the subunit NDUFS2. The mouse Asn232 of the NDUFS2 protein corresponded to the Asn265 in the human NDUFS2 subunit.

We hypothesised that the *MT-ND1* p.Tyr277His missense variant stabilizes the inactive form of CI. Substitution of hydrophobic Tyr to hydrophilic His at position 277 may alter CI structure and therefore formation of SCs is disrupted. The reduction of ubiquinone still occurs, since the CI+III activity is only partially disturbed. Due to decreased ability to form SCs, cells preferred alternative electrons to flow through CII (elevated CII and CII+III activities) and probably also through other pathways which we did not study (e.g. sulfide quinone oxidoreductase (SQOR)). Secondly, because of stabilizing effect of the variant on the inactive form of CI, where ubiquinone does not bind to the ubiquinone binding cavity of CI, CI-containing SC is not assembled. Instead, other pathways with a source of electrons for ubiquinone (CII, SQOR) are boosted and activities of remaining respiratory chain complexes are elevated. Thirdly, the mutation could destabilize the natural equilibrium between mentioned CI-containing SC states, by increasing the population of the inactive conformation and perhaps altering the natural mechanism of allosteric regulation of the respiration chain activity.

In our study [61], we also showed that mitochondrial energy-generating capacity (MEGS) analysis may serve as a good indicator for CI deficiency and may help to advance the diagnosis. Moreover, in fibroblasts carrying a mutation in the *MT-ND* gene, MEGS seems to be more sensitive compared to spectrophotometry [61]. As was demonstrated above, combining several biochemical methods may improve our understanding of the impact of individual mutations of *MT-ND* genes on mitochondrial bioenergetics and helps us to confirm the pathogenicity of novel variants.

4.3 Results and discussion related to the aim C) Study of SCs in patients with rare metabolic disorders

Rákosníková Tereza, Kelifová Silvie, Štufková Hana, Lišková Petra, Kousal Bohdan, Martínek Václav, Honzík Tomáš, Hansíková Hana, Tesařová Markéta. "A rare variant m.4135T>C in the *MT-ND1* gene leads to LHON and altered OXPHOS supercomplexes." (manuscript prepared for submission)

As discussed in chapter 4.2.1 Characterisation of mitochondrial disorders caused by a mutation in *MT-ND* genes including two novel variants

Zdražilová Lucie, **Rákosníková Tereza**, Ondrušková Nina, Pasák Michael, Vanišová Marie, Volfová Nikol, Honzík Tomáš, Thiel Christian, Hansíková Hana. „Metabolic adaptation of human skin fibroblasts to ER stress caused by glycosylation defect in PMM2-CDG." (manuscript prepared for submission)

4.3.1 Study of SCs in patients with congenital disorders of glycosylation caused by a mutation in the PMM2 gene

In three patients (P1-PMM2, P7-PMM2 and P8-PMM2) carrying different mutations in the *PMM2* (phosphomannomutase 2) gene, a study of the representation of SCs in fibroblasts was performed. In all three analysed patients, an increased level of CI-containing SCs together with a decreased level of free CI was found. Moreover, compared to controls, in P7-PMM2 and P8-PMM2, analysis of CII showed a decreased level of free SDHA subunit and SDHA+SDHB subcomplexes, demonstrating the tendency of CII to undergo more efficient or faster assembly. The study of the formation of SCs is primarily performed on cells derived from mitochondrial patients or model cells or organisms, but not much research has been done on other metabolic diseases. Congenital disorders of glycosylation (CDG) are a rare group of disorders caused by defective glycosylation of proteins and/or lipids. The most common type of CDG is a disorder caused by a mutation in the enzyme PMM2. This enzyme catalyses the conversion of mannose-6-phosphate to mannose-1-phosphate which activates saccharide to downstream glycosylation processes. In U2OS human bone osteosarcoma epithelial cells, ER stress induced by glucose deprivation leads to increased formation of SCs [91]. Since ER is a key compartment of glycosylation and ER stress occurs in CDG patients [92], we decided to study SCs formation in PMM2 patients.

The exact function of SCs is still debated, but it is assumed that the formation of SCs should lead to higher effectivity of OXPHOS functions. In PMM2 patients, an increased CI-containing SCs formation shows the tendency of the OXPHOS system to form and prefer higher structures, probably to generate more efficient systems. It is well known that cells can quickly switch between glycolysis and OXPHOS in response to the lack of nutrients. In PMM2 patients, it is hypothesised that glucose is probably mainly used for conversion to the mannose-6-phosphate rather than as a substrate for glycolysis. Moreover, nutrient deprivation and disruption of glycosylation could lead to the accumulation of unfolded or misfolded proteins in the ER (activation of the unfolded protein response), resulting in ER stress. During the ER stress, activation of the PERK-elF2α-ATF4 pathway occurs, leading to increased expression of SCAF1 and increased formation of SCs [91]. We showed that ER stress coupled with the PERK-elF2α-ATF4 pathway activation occurs in PMM2-CDG patients and results in increased formation of CI-containing SCs. Moreover, significantly increased activities of CI and CII were found in PMM2 patients (unpublished results – Zdražilová et al[6]) together with a decreased level of α-ketoglutarate in the culture medium. Decreased amount of α-ketoglutarate and increased activities of CII lead to an altered ratio of $NAD^+/NADH+H^+$ and $FAD/FADH_2$, respectively. Besides, other metabolic pathways upstream of OXPHOS, were modified in PMM2-deficient fibroblasts (decreased glycolysis, decreased level of pyruvate in culture medium, and decreased activity of pyruvate dehydrogenase and CS) (unpublished results – Zdražilová et al[6]). Those metabolic changes could lead to a shifting of OXPHOS from the isolated complex toward the SCs to create a more efficient system that can compensate for the lack of ATP.

[6] Zdražilová Lucie, Rákosníková Tereza, Ondrušková Nina, Pasák Michael, Vanišová Marie, Volfová Nikol, Honzík Tomáš, Thiel Christian, Hansíková Hana. „Metabolic adaptation of human skin fibroblasts to ER stress caused by glycosylation defect in PMM2-CDG.“manuscript in preparation

5 CONCLUSIONS

Molecular diagnosis of patients with suspicion of mitochondrial disorders can be realized for many cases through next-generation sequencing of blood DNA, but the use of patient tissues and an integrated, multidisciplinary approach is pivotal for the diagnosis of more challenging cases. Furthermore, an analysis of clinically relevant tissues from affected individuals remains crucial for understanding the molecular mechanisms underlying mitochondrial pathology. For the thesis, three specific aims were defined to confirm the pathogenicity of newly found variants or genes in our cohort of patients and to characterise their pathology mechanism.

The first aim: a study of the role of ACBD3 protein in mitochondria and Golgi in HEK293 and HeLa cells

- three ACBD3-KO clones in HEK293 cell line and one ACBD3-KO clone in HeLa cell line were constructed
- ACBD3 has no essential function in mitochondria in HEK293 and HeLa cells
- the pathogenicity of the variant in the *ACBD3* gene in our patient was not confirmed
- ACBD3 protein absence leads to altered Golgi structure but the glycosylation pattern of LAMP2 glycoprotein remains unchanged as well as the amount of selected Golgi proteins participating in Golgi structure maintenance
- ACBD3 protein absence leads to a decreased level of CoQ_9, but the mechanism of how ACBD3 protein may affect the level of CoQ_9 remains unclear
- ACBD3 protein has a role in ceramide transport from ER to Golgi as a substrate for SMS, but not for GCS

The second aim: description of the impact of novel variants in mtDNA-encoded genes on mitochondrial energetic metabolism

- two novel variants m.4135T>C (p.Tyr277His) in *MT-ND1* and m.13091T>C (p.Met252Thr) in the *MT-ND5* gene were characterised and pathogenic mechanism on CI was proposed
- in both patients, the pathogenicity of the variants was successfully confirmed and partially supported by experimental data
- on a clinical and biochemical level, a cohort of Czech and Slovak patients with CI deficiency caused by mutations in the mtDNA-encoded structural subunit for CI was described

The third aim: study of SCs in patients with rare metabolic disorders

- In P14 with mutation m.4135T>C (p.Tyr277His) in *MT-ND1* albeit the CI is successfully assembled, the activity of CI and CI+III and the ability to form SCs is decreased probably due to the stabilisation role of the mutation in the inactive form of CI.
- In the PMM2 patients, an increased formation of CI-containing SCs and a tendency of CII to undergo more efficient or faster assembly were shown. These results helped to a better understanding of bioenergetics status in PMM2 patients.

6 REFERENCES

[1] J. Rahman, S. Rahman, The Lancet 391 (2018) 2560–2574.
[2] M. Protasoni, M. Zeviani, Int. J. Mol. Sci. 22 (2021) 586.
[3] R. Acin-Perez, J.A. Enriquez, Biochim. Biophys. Acta BBA - Bioenerg. 1837 (2014) 444–450.
[4] R. Acín-Pérez, P. Fernández-Silva, M.L. Peleato, A. Pérez-Martos, J.A. Enriquez, Mol. Cell 32 (2008) 529–539.
[5] D.R. Thorburn, J. Inherit. Metab. Dis. 27 (2004) 349–362.
[6] G.S. Gorman, A.M. Schaefer, Y. Ng, N. Gomez, E.L. Blakely, C.L. Alston, C. Feeney, R. Horvath, P. Yu-Wai-Man, P.F. Chinnery, R.W. Taylor, D.M. Turnbull, R. McFarland, Ann. Neurol. 77 (2015) 753–759.
[7] S.L. Stenton, M. Shimura, D. Piekutowska-Abramczuk, P. Freisinger, F. Distelmaier, J.A. Mayr, C. Makowski, B. Büchner, B. Alhaddad, C.L. Alston, A. Ardissone, R. Ban, I. Barić, R. Berutti, T. Brunet, E. Ciara, D. Deen, J. Gagneur, D. Ghezzi, M. Gusic, T.B. Haack, M. Hempel, R.A. Husain, D. Karall, S. Kölker, U. Kotzaeridou, T. Klopstock, R. Kopajtich, V. Konstantopoulou, S. Liez, D. Lenz, A.Z. Lim, H. Mandel, R. McFarland, W. Müller-Felber, G. Muñoz-Pujol, A. Ohtake, Y. Okazaki, R. Olsen, E. Pronicka, A. Pyle, A. Ribes, D. Rokicki, R. Santer, M. Schiff, M. Schuelke, D. Smirnov, W. Sperl, T. Strom, F. Tort, P. Tsygankova, R. van Coster, P. Verloo, J.-C. von Kleist-Retzow, E. Wilichowski, T. Wolstein, M. Xu, V. Yépez, M. Zech, S. Wortmann, M. Wagner, C. Lamperti, R.W. Taylor, F. Fang, A. Rötig, K. Murayama, T. Meitinger, H. Prokisch, Diagnosing Pediatric Mitochondrial Disease: Lessons from 2,000 Exomes, Genetic and Genomic Medicine, 2021.
[8] S. Wortmann, J. Mayr, J. Nuoffer, H. Prokisch, W. Sperl, Neuropediatrics 48 (2017) 309–314.
[9] S.E. Horvath, G. Daum, Prog. Lipid Res. 52 (2013) 590–614.
[10] M. Bosch, M. Marí, S.P. Gross, J.C. Fernández-Checa, A. Pol, Traffic 12 (2011) 1483–1489.
[11] C. García-Ruiz, M. Marí, A. Colell Riera, A. Morales, F. Caballero, J. Montero, O. Terrones, G. Basañez, J.C. Fernández-Checa, (2009).
[12] M. Marí, A. Morales, A. Colell, C. García-Ruiz, J.C. Fernández-Checa, Redox Biol. 3 (2014) 100–108.
[13] J. Montero, M. Mari, A. Colell, A. Morales, G. Basañez, C. Garcia-Ruiz, J.C. Fernández-Checa, Biochim. Biophys. Acta BBA - Bioenerg. 1797 (2010) 1217–1224.
[14] A. Colell, A. Fernández, J.C. Fernández-Checa, J. Bioenerg. Biomembr. 41 (2009) 417.
[15] L.A. Martin, B.E. Kennedy, B. Karten, J. Bioenerg. Biomembr. 48 (2016) 137–151.
[16] P. Elustondo, L.A. Martin, B. Karten, Biochim. Biophys. Acta BBA - Mol. Cell Biol. Lipids 1862 (2017) 90–101.
[17] J.M. Gerhold, Ş. Cansiz-Arda, M. Lõhmus, O. Engberg, A. Reyes, H. van Rennes, A. Sanz, I.J. Holt, H.M. Cooper, J.N. Spelbrink, Sci. Rep. 5 (2015).
[18] J. He, H.M. Cooper, A. Reyes, M. Di Re, H. Sembongi, T.R. Litwin, J. Gao, K.C. Neuman, I.M. Fearnley, A. Spinazzola, J.E. Walker, I.J. Holt, Nucleic Acids Res. 40 (2012) 6109–6121.
[19] J. He, C.-C. Mao, A. Reyes, H. Sembongi, M. Di Re, C. Granycome, A.B. Clippingdale, I.M. Fearnley, M. Harbour, A.J. Robinson, S. Reichelt, J.N. Spelbrink, J.E. Walker, I.J. Holt, J. Cell Biol. 176 (2007) 141–146.
[20] S. Peralta, S. Goffart, S.L. Williams, F. Diaz, S. Garcia, N. Nissanka, E. Area-Gomez, J. Pohjoismäki, C.T. Moraes, J. Cell Sci. 131 (2018) jcs217075.
[21] R. Desai, A.E. Frazier, R. Durigon, H. Patel, A.W. Jones, I. Dalla Rosa, N.J. Lake, A.G. Compton, H.S. Mountford, E.J. Tucker, A.L.R. Mitchell, D. Jackson, A. Sesay, M. Di Re, L.P. van den Heuvel, D. Burke, D. Francis, S. Lunke, G. McGillivray, S. Mandelstam, F. Mochel, B. Keren, C. Jardel, A.M. Turner, P. Ian Andrews, J. Smeitink, J.N. Spelbrink, S.J. Heales, M. Kohda, A. Ohtake, K. Murayama, Y. Okazaki, A. Lombès, I.J. Holt, D.R. Thorburn, A. Spinazzola, Brain 140 (2017) 1595–1610.
[22] M. Fujimoto, T. Hayashi, in: K.W. Jeon (Ed.), Int. Rev. Cell Mol. Biol., Academic Press, 2011, pp. 73–117.
[23] A. Duarte, C. Poderoso, M. Cooke, G. Soria, F. Cornejo Maciel, V. Gottifredi, E.J. Podestá, PLoS ONE 7 (2012) e45829.
[24] K.-S.C. Marriott, M. Prasad, V. Thapliyal, H.S. Bose, J. Pharmacol. Exp. Ther. 343 (2012) 578–586.
[25] J.-P. Andersen, J. Zhang, H. Sun, X. Liu, J. Liu, J. Nie, Y. Shi, Mol. Metab. 42 (2020) 101055.
[26] Q. Shi, J. Chen, X. Zou, X. Tang, Front. Cell Dev. Biol. 10 (2022).
[27] F. Giordano, Biochem. Soc. Trans. 46 (2018) 437–452.
[28] D.M. Stocco, Mol. Cell. Endocrinol. 191 (2002) 19–25.
[29] J. Liu, M.B. Rone, V. Papadopoulos, J. Biol. Chem. 281 (2006) 38879–38893.
[30] M.B. Rone, A.S. Midzak, L. Issop, G. Rammouz, S. Jagannathan, J. Fan, X. Ye, J. Blonder, T. Veenstra, V. Papadopoulos, Mol. Endocrinol. 26 (2012) 1868–1882.

[31] M.B. Rone, J. Fan, V. Papadopoulos, Biochim. Biophys. Acta BBA - Mol. Cell Biol. Lipids 1791 (2009) 646–658.
[32] A. Hubstenberger, N. Merle, R. Charton, G. Brandolin, D. Rousseau, J. Bioenerg. Biomembr. 42 (2010) 143–150.
[33] B. Gilquin, E. Taillebourg, N. Cherradi, A. Hubstenberger, O. Gay, N. Merle, N. Assard, M.-O. Fauvarque, S. Tomohiro, O. Kuge, J. Baudier, Mol. Cell. Biol. 30 (2010) 1984–1996.
[34] H. Li, Z.-X. Yao, B. Degenhardt, G. Teper, V. Papadopoulos, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 1267–1272.
[35] N. Jamin, J.-M. Neumann, M.A. Ostuni, T.K.N. Vu, Z.-X. Yao, S. Murail, J.-C. Robert, C. Giatzakis, V. Papadopoulos, J.-J. Lacapère, Mol. Endocrinol. 19 (2005) 588–594.
[36] J. Fantini, C. Di Scala, C.J. Baier, F.J. Barrantes, Chem. Phys. Lipids 199 (2016) 52–60.
[37] M. Culty, H. Li, N. Boujrad, H. Amri, B. Vidic, J.M. Bernassau, J.L. Reversat, V. Papadopoulos, J. Steroid Biochem. Mol. Biol. 69 (1999) 123–130.
[38] F. Delavoie, H. Li, M. Hardwick, J.-C. Robert, C. Giatzakis, G. Péranzi, Z.-X. Yao, J. Maccario, J.-J. Lacapère, V. Papadopoulos, Biochemistry 42 (2003) 4506–4519.
[39] J. Fantini, C. Di Scala, L.S. Evans, P.T.F. Williamson, F.J. Barrantes, Sci. Rep. 6 (2016).
[40] V. Anantharaman, L. Aravind, Genome Biol. 3 (2002) research0023.1.
[41] M. Islinger, J.L. Costello, S. Kors, E. Soupene, T.P. Levine, F.A. Kuypers, M. Schrader, Biochim. Biophys. Acta Mol. Cell Res. 1867 (2020) 118675.
[42] L.F.S. Mendes, A.J. Costa-Filho, FEBS Lett. 596 (2022) 973–990.
[43] M. Uhlen, L. Fagerberg, B.M. Hallstrom, C. Lindskog, P. Oksvold, A. Mardinoglu, A. Sivertsson, C. Kampf, E. Sjostedt, A. Asplund, I. Olsson, K. Edlund, E. Lundberg, S. Navani, C.A.-K. Szigyarto, J. Odeberg, D. Djureinovic, J.O. Takanen, S. Hober, T. Alm, P.-H. Edqvist, H. Berling, H. Tegel, J. Mulder, J. Rockberg, P. Nilsson, J.M. Schwenk, M. Hamsten, K. von Feilitzen, M. Forsberg, L. Persson, F. Johansson, M. Zwahlen, G. von Heijne, J. Nielsen, F. Ponten, Science 347 (2015) 1260419–1260419.
[44] The UniProt Consortium, Nucleic Acids Res. 47 (2019) D506–D515.
[45] J.X. Binder, S. Pletscher-Frankild, K. Tsafou, C. Stolte, S.I. O'Donoghue, R. Schneider, L.J. Jensen, Database J. Biol. Databases Curation 2014 (2014).
[46] S. Rath, R. Sharma, R. Gupta, T. Ast, C. Chan, T.J. Durham, R.P. Goodman, Z. Grabarek, M.E. Haas, W.H.W. Hung, P.R. Joshi, A.A. Jourdain, S.H. Kim, A.V. Kotrys, S.S. Lam, J.G. McCoy, J.D. Meisel, M. Miranda, A. Panda, A. Patgiri, R. Rogers, S. Sadre, H. Shah, O.S. Skinner, T.-L. To, M.A. Walker, H. Wang, P.S. Ward, J. Wengrod, C.-C. Yuan, S.E. Calvo, V.K. Mootha, Nucleic Acids Res. 49 (2021) D1541–D1547.
[47] M. Sohda, Y. Misumi, A. Yamamoto, A. Yano, N. Nakamura, Y. Ikehara, J. Biol. Chem. 276 (2001) 45298–45306.
[48] H. Li, Mol. Endocrinol. 15 (2001) 2211–2228.
[49] X. Yue, M. Bao, R. Christiano, S. Li, J. Mei, L. Zhu, F. Mao, Q. Yue, P. Zhang, S. Jing, J.E. Rothman, Y. Qian, I. Lee, FEBS Lett. 591 (2017) 2793–2802.
[50] J. Liao, Y. Guan, W. Chen, C. Shi, D. Yao, F. Wang, S.M. Lam, G. Shui, X. Cao, J. Mol. Cell Biol. 11 (2019) 107–117.
[51] Y. Shinoda, K. Fujita, S. Saito, H. Matsui, Y. Kanto, Y. Nagaura, K. Fukunaga, S. Tamura, T. Kobayashi, FEBS Lett. 586 (2012) 3024–3029.
[52] R.T. Sherpa, C. Fiore, K.S. Moshal, A. Wadsworth, M.W. Rudokas, S.R. Agarwal, R.D. Harvey, Physiol. Rep. 9 (2021) e15015.
[53] J. Liu, H. Li, V. Papadopoulos, J. Steroid Biochem. Mol. Biol. 85 (2003) 275–283.
[54] J.H. Cheah, S.F. Kim, L.D. Hester, K.W. Clancy, S.E. Patterson, V. Papadopoulos, S.H. Snyder, Neuron 51 (2006) 431–440.
[55] Y. Okazaki, Y. Ma, M. Yeh, H. Yin, Z. Li, K. Yeh, J. Glass, Am. J. Physiol.-Gastrointest. Liver Physiol. 302 (2012) G1180–G1190.
[56] J. Sasaki, K. Ishikawa, M. Arita, K. Taniguchi, EMBO J. 31 (2012) 754–766.
[57] A. Greninger, G. Knudsen, M. Betegon, A. Burlingame, J. DeRisi, J. Virol. 86 (2012) 3605–3616.
[58] J.A. McPhail, E.H. Ottosen, M.L. Jenkins, J.E. Burke, Structure 25 (2017) 121–131.
[59] K. Ishikawa-Sasaki, S. Nagashima, K. Taniguchi, J. Sasaki, J. Virol. 92 (2018) e01952-17.
[60] T. Daňhelovská, L. Zdražilová, H. Štufková, M. Vanišová, N. Volfová, J. Křížová, O. Kuda, J. Sládková, M. Tesařová, Int. J. Mol. Sci. 22 (2021) 7270.
[61] T. Danhelovska, H. Kolarova, J. Zeman, H. Hansikova, M. Vaneckova, L. Lambert, V. Kucerova-Vidrova, K. Berankova, T. Honzik, M. Tesarova, BMC Pediatr. 20 (2020) 41.
[62] R. Guo, S. Zong, M. Wu, J. Gu, M. Yang, Cell 170 (2017) 1247-1257.e12.
[63] C.H. Rodrigues, D.E. Pires, D.B. Ascher, Nucleic Acids Res. 46 (2018) W350–W355.

[64] H. Ashkenazy, S. Abadi, E. Martz, O. Chay, I. Mayrose, T. Pupko, N. Ben-Tal, Nucleic Acids Res. 44 (2016) W344–W350.
[65] W.L. Miller, Mol. Cell. Endocrinol. (2013) 12.
[66] A. Midzak, M. Rone, Y. Aghazadeh, M. Culty, V. Papadopoulos, Mol. Cell. Endocrinol. 336 (2011) 70–79.
[67] R. Desai, M. Campanella, Br. J. Pharmacol. 176 (2019) 4284–4292.
[68] A. Midzak, V. Papadopoulos, Front. Endocrinol. 7 (2016).
[69] V. Papadopoulos, P. Guarneri, K.E. Kreuger, A. Guidotti, E. Costa, Proc. Natl. Acad. Sci. 89 (1992) 5113–5117.
[70] N. Boujrad, J.R. Hudson, V. Papadopoulos, Proc. Natl. Acad. Sci. 90 (1993) 5728–5731.
[71] M. Garnier, N. Boujrad, S.O. Ogwuegbu, J.R. Hudson, V. Papadopoulos, J. Biol. Chem. 269 (1994) 22105–22112.
[72] V. Papadopoulos, A.S. Brown, J. Steroid Biochem. Mol. Biol. 53 (1995) 103–110.
[73] J. Fan, X. Li, L. Issop, M. Culty, V. Papadopoulos, Mol. Endocrinol. 30 (2016) 763–782.
[74] M.E. Bekier, L. Wang, J. Li, H. Huang, D. Tang, X. Zhang, Y. Wang, Mol. Biol. Cell 28 (2017) 2833–2842.
[75] R. Saiki, A. Nagata, T. Kainou, H. Matsuda, M. Kawamukai, FEBS J. 272 (2005) 5606–5622.
[76] E.L. Huttlin, R.J. Bruckner, J.A. Paulo, J.R. Cannon, L. Ting, K. Baltier, G. Colby, F. Gebreab, M.P. Gygi, H. Parzen, J. Szpyt, S. Tam, G. Zarraga, L. Pontano-Vaites, S. Swarup, A.E. White, D.K. Schweppe, R. Rad, B.K. Erickson, R.A. Obar, K.G. Guruharsha, K. Li, S. Artavanis-Tsakonas, S.P. Gygi, J.W. Harper, Nature 545 (2017) 505–509.
[77] A.L. Greninger, G.M. Knudsen, M. Betegon, A.L. Burlingame, J.L. DeRisi, MBio 4 (2013) e00098-13.
[78] K. Kumagai, K. Hanada, FEBS Lett. 593 (2019) 2366–2377.
[79] T. Yamaji, K. Hanada, PLoS ONE 9 (2014) e88124.
[80] B.E. Housden, M. Muhar, M. Gemberling, C.A. Gersbach, D.Y.R. Stainier, G. Seydoux, S.E. Mohr, J. Zuber, N. Perrimon, Nat. Rev. Genet. 18 (2017) 24–40.
[81] A.M. Zimmer, Y.K. Pan, T. Chandrapalan, R.W.M. Kwong, S.F. Perry, J. Exp. Biol. 222 (2019) jeb175737.
[82] S. Shanske, J. Coku, J. Lu, J. Ganesh, S. Krishna, K. Tanji, E. Bonilla, A.B. Naini, M. Hirano, S. DiMauro, Arch. Neurol. 65 (2008) 368–372.
[83] A. Sudo, S. Honzawa, I. Nonaka, Y. Goto, J. Hum. Genet. 49 (2004) 92–96.
[84] M. Chol, J. Med. Genet. 40 (2003) 188–191.
[85] V. Dvorakova, H. Kolarova, M. Magner, M. Tesarova, H. Hansikova, J. Zeman, T. Honzik, Mol. Genet. Metab. 118 (2016) 288–295.
[86] S.C. Lim, J. Hroudová, N.J. Van Bergen, M.I.G. Lopez Sanchez, I.A. Trounce, M. McKenzie, FASEB J. Off. Publ. Fed. Am. Soc. Exp. Biol. 30 (2016) 2236–2248.
[87] J. Marco-Brualla, S. Al-Wasaby, R. Soler, E. Romanos, B. Conde, R. Justo-Méndez, J.A. Enríquez, P. Fernández-Silva, L. Martínez-Lostao, M. Villalba, R. Moreno-Loshuertos, A. Anel, Cancers 11 (2019) 1027.
[88] M. Xu, R. Kopajtich, M. Elstner, H. Li, Z. Liu, J. Wang, H. Prokisch, F. Fang, Mitochondrion 62 (2022) 13–23.
[89] S. Castellana, T. Biagini, F. Petrizzelli, L. Parca, N. Panzironi, V. Caputo, A.L. Vescovi, M. Carella, T. Mazza, Nucleic Acids Res. 49 (2021) D1282–D1288.
[90] A.-N.A. Agip, J.N. Blaza, H.R. Bridges, C. Viscomi, S. Rawson, S.P. Muench, J. Hirst, Nat. Struct. Mol. Biol. 25 (2018) 548–556.
[91] E. Balsa, M.S. Soustek, A. Thomas, S. Cogliati, C. García-Poyatos, E. Martín-García, M. Jedrychowski, S.P. Gygi, J.A. Enriquez, P. Puigserver, Mol. Cell 74 (2019) 877-890.e6.
[92] P. Yuste-Checa, A.I. Vega, C. Martín-Higueras, C. Medrano, A. Gámez, L.R. Desviat, M. Ugarte, C. Pérez-Cerdá, B. Pérez, PLOS ONE 12 (2017) e0179456.

7 LIST OF PUBLICATIONS

Publications *in extenso,* that constitute the basis of the PhD thesis

a) with impact factor (IF)

Tereza Danhelovska, Hana Kolarova, Jiri Zeman, Hana Hansikova, Manuela Vaneckova, Lukas Lambert, Vendula Kucerova-Vidrova, Kamila Berankova, Tomas Honzik, and Marketa Tesarova. "Multisystem Mitochondrial Diseases Due to Mutations in MtDNA-Encoded Subunits of Complex I." BMC Pediatrics 20, no. 1 (January 29, 2020): 41. https://doi.org/10.1186/s12887-020-1912-x. (IF = 2.765)

Tereza Daňhelovská, Lucie Zdražilová, Hana Štufková, Marie Vanišová, Nikol Volfová, Jana Křížová, Ondřej Kuda, Jana Sládková, and Markéta Tesařová. 2021. "Knock-Out of ACBD3 Leads to Dispersed Golgi Structure, but Unaffected Mitochondrial Functions in HEK293 and HeLa Cells." International Journal of Molecular Sciences 22 (14): 7270. https://doi.org/10.3390/ijms22147270. (IF = 5.924)

Rákosníková Tereza, Kelifová Silvie, Štufková Hana, Lišková Petra, Kousal Bohdan, Martínek Václav, Honzík Tomáš, Hansíková Hana, Tesařová Markéta. "A rare variant m.4135T>C in the *MT-ND1* gene leads to LHON and altered OXPHOS supercomplexes." (manuscript prepared for submission)

Zdražilová Lucie, **Rákosníková Tereza**, Ondrušková Nina, Pasák Michael, Vanišová Marie, Volfová Nikol, Honzík Tomáš, Thiel Christian, Hansíková Hana. Metabolic adaptation of human skin fibroblasts to ER stress caused by glycosylation defect in PMM2-CDG. (manuscript prepared for submission)

Other publications *in extenso*

a) with impact factor (IF)

Vanisova, M, D Burska, J Krizova, **T Danhelovska**, Z Dosoudilova, J Zeman, L Stiburek, and H Hansikova. "Stable COX17 Downregulation Leads to Alterations in Mitochondrial Ultrastructure, Decreased Copper Content and Impaired Cytochrome c Oxidase Biogenesis in HEK293 Cells." Folia Biologica, 2019. (IF= 0.69)

Vanisova, Marie, Hana Stufkova, Michaela Kohoutova, **Tereza Rakosnikova**, Jana Krizova, Jiri Klempir, Irena Rysankova, Jan Roth, Jiri Zeman, and Hana Hansikova. 2022. "Mitochondrial Organization and Structure Are Compromised in Fibroblasts from Patients with Huntington's Disease." Ultrastructural Pathology, August, 1–14. https://doi.org/10.1080/01913123.2022.2100951. (IF = 1.385)