Opponent's review of the PhD thesis of Mgr. Tereza Rákosníková – Study of etiopathology of mitochondrial disorders.

The submitted thesis has been written based on author's research work at the Department of Paediatrics and Inherited Metabolic Disorders, First Faculty of Medicine, under the supervision of Ing. Markéta Tesařová, PhD. The department has been very well known to the scientific community in the field for outstanding and high impact research of mitochondrial disorders. A look at Student information system of Charles University reveals that Mgr. Tereza Rákosníková has been taking part in the scientific program of this department since at least 2013. At that time, she worked on her bachelor's thesis, later she completed her master thesis there, and this experience is apparent in the quality of her doctoral thesis. She is very well oriented in the field and can therefore tackle the problems outside the direct scope of mithopathies.

The thesis is based on four publications in international journals and Mgr. Tereza Rákosníková is listed as the first author of three of them (two are still in the form of manuscripts). The thesis also mentions two other publications that are not directly related to the doctoral thesis, but in which Mgr. Tereza Rákosníková participated. This proves that she was not idle during her doctoral studies. The author chose the traditional unabridged form for her thesis, so it consists of an Introduction, Material and methods, Results and Discussion, as well as a summary of results and a list of literature. The publications on which the thesis is based have been attached. The work is written in good English, as far as I can judge.

In contrast to the general title of the thesis, the author focused on the possible role of a specific protein in mitochondrial disorders on one hand and on the other hand on specific phenomena at the level of energy production, aggregation of OXFOS complexes and subcellular morphology. This is an attractive topic with a significant link to clinical practice. The conclusions reached in the papers, which are the basis of the theses, certainly represent the result of the efforts of a wide team of people, but in the text of her thesis the author states exactly what methods she mastered and what was her contribution to the data obtained and to the preparation of the manuscript.

The theoretical introduction is a comprehensive summary of the researched issue based on a 27 pages long list of literature. It is in itself quite extensive, almost 40 pages of text, but it does not get bogged down in trivialities and repetition of textbook facts. Its scope proves the author's outstanding

orientation in the issue and mastering of theoretical foundations. Summaries for all parts of the thesis concisely present the most important results obtained.

Formally, the work is very good. It contains graphically attractive and illustrative figures that contribute to the understanding of the text. Also, experimental pictures are of good quality, see comments on TEM photos. When reading the text, I did not find any typos or grammatical errors. I have a few more or less formal comments on the work, which I have summarized in six points.

1. On page 15 it is stated that complex I consists of 44 subunits. In the thesis, the quoted paper of Kampyut and Sazanov lists 45 subunits.

2. I am interested in the origin of *Figure 2: The proposed catalytic cycle of Complex I.* on page 18. In the article given at the end of the legend, it is not to be found in this form. Did the author create it herself based on the materials in the cited article? One sentence was also not clear to me: *Releasing of ubiquinol leads to the uptake of protons, reduction of NADH… etc.* Is it really a reduction?

3. *Figure 5: Respiratory complexes and supercomplexes composition* on page 27 is not entirely clear. According to part (b), 2 dimers $CIII_2$ (black arrow) move to the megacomplex, but only one is listed.

4. On page 41 it is stated: the *amount of cholesterol in the mitochondria is approx. 40 times lower compared to the plasma membrane.* This refers to mitochondrial membranes when compared to plasma membrane or entire mitochondria?

5. At first, I was disappointed a bit looking at the TEM microphotos on page 73. In printed form, they are rather small, and I found it difficult to discern anything on them. Fortunately, I found that in PDF format, images can be enlarged and turn out to be of good quality, contrasting and informative. At the same time, however, the question occurred to me, how are subcellular structures evaluated, what is normal and what is abnormal? Where is the boundary between these two states and can it be quantified?

6. On page 93 I came across a sentence: *Histochemistry in the skeletal muscle biopsy revealed focal subsarcolemmal accumulation of the SDH (succinate dehydrogenase) reaction product in…. etc.* Does this mean that fumarate is accumulating here, and if so, why?

The wide scope of the submitted work naturally raises a lot of questions. However, since it is not possible to discuss them all, I choose several that I find especially relevant.

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1. Does the author have any idea why Complex II does not occur in supercomplexes? Is anything known about the possible interactions of OXPHOS complexes with other FAD-dependent dehydrogenases (GPDH, DHODH or ETFDH)?

2. On page 40 the author states: *The exchange of material through the contact site is spatially segregated but mutually coordinated with vesicular transport.* Is something known about this mutual coordination; I can think of nothing.

3. How do you explain the differences between earlier findings of ACBD3 protein in the mitochondria of HEK293 cells? How come it wasn't found there now? Can HEK293 cells really differ so significantly from line to line when they are used as an experimental model from 1973? Have you tested HeLa cells before? Is it possible to compare old and new HEK293 cells in other parameters as well?

4. The question of function and synthesis of Coenzyme Q_9 is interesting, could it be an intermediate in synthesis or degradation of Coenzyme Q_{10}? I did a bit of search, and there are indeed many unknown in the synthetic pathway of CoenzymeQ_{10}.

5. I was intrigued by the fact that the author did not find a significant correlation between the enzymatic activities of OXPHOS complexes and the degree of heteroplasmy in muscle tissue. Is there an explanation for this, or is it a common phenomenon?

6. In the last part of the thesis (page 100) the author mentions the reduced content of subunits SDHA and SDHB in patients with glycosylation disorder and attributes it to an accelerated or more efficient assembly of complex II. Is there no other explanation? In the thesis I did not find evidence for the increased content of the whole complex II.

In conclusion, I am convinced that the dissertation of Mgr. Tereza Rákosníková is an above-average work and brings several new and significant findings that will certainly contribute to a deeper understanding of the etiopathology of mitochondrial disorders and at the same time to a deeper understanding of a wider range of processes at the subcellular level. These results can ultimately have a significant clinical impact.

In my opinion, Mgr. Tereza Rákosníková proved in her dissertation that she can plan, perform, and evaluate scientific experiments and is therefore capable of independent scientific work. Therefore, I recommend that she be awarded a PhD based on this thesis.

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