



Apr 6, 2018, New Orleans

To Whom it may concern

The work is elegant, the candidate shows familiarity with both clinical and laboratory sciences.

The introduction is detailed and correct, the methods are clear. I have to add that it's really difficult to follow the different projects though, due to the thesis structure which divides the different chapters into separate sections within the results and discussion sections, instead of discussing one topic as a whole.

The candidate participated in many projects and publications and was first and second author on several papers as well.

I focus my questions on these papers.

Developmental changes of COX, CS, SQR and mtDNA in human foetal tissues

1. The candidate found a decrease in COX and CS activity after the 22nd week in liver in parallel with increasing expression levels. What could be the physiologic relevance of the decreased COX activity around 22 weeks in the liver, (while expression changes are opposite in direction)? Could this mean a signaling function to induce transcription activity? Why would COX have a specific role like that, and not other complexes?
2. How can CS activity decrease while mtDNA amount is stable (or increase), usually CS activity is a measure of mtDNA presence and activity and mitochondrial volume.
3. The enzyme activities normalized to mtDNA showed a downward trend throughout lifetime in the male group (not in females). This, rather unexpected finding, according to the candidate, could indicate that skeletal muscle loses its efficiency to produce enzyme specific activity per mtDNA unit with age. Did the candidate look into the possibility of acquired mtDNA mutations which could lead to decreasing activities while the mtDNA amount is stable throughout the years?
4. The mtDNA content tended to get increased in males with age. Could this be relative, in other words, with aging muscle mass could decrease (especially protein content), therefore mtDNA content compared to protein concentration

could be a misleading measurement? Also I don't understand the hypothesis with testosterone, I thought that that level usually decreases with aging.


Extraocular features in LHON

This is a very important observation, I personally also share with the candidate. I would like to suggest though, that additional to the whole exome sequencing and mtDNA sequencing the candidate should evaluate a second tissue for second mtDNA alterations. Did the candidate rule out mtDNA deletions in muscle, and any mtDNA variants in urine sediments or fibroblasts (or muscle)?

Peripapillary microcirculation in LHON

Very elegant measurement. One thing was not clear for me in the description; in the patient treated with Idebenone, the peripapillary alterations were identical with the others before, or also after Idebenone treatment? What does the candidate think about the relevance of this finding with regards to the potential of Idebenone in long term treatment efficacy, and the use of the method to monitor that?

I was very satisfied with the overall work and wish the candidate good luck with her defense.



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