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Jury Member Report – Doctor of Philosophy thesis.

Name of Candidate: Aleksandra Mitina

PhD Program: Life Sciences

Title of Thesis: Role of breast milk lipid composition in postnatal brain development

Supervisor: Professor Philipp Khaitovich

Name of the Reviewer:

I confirm the absence of any conflict of interest (Alternatively, Reviewer can formulate a possible conflict)	**Signature:** **Date: 02-12-2020**

The purpose of this report is to obtain an independent review from the members of PhD defense Jury before the thesis defense. The members of PhD defense Jury are asked to submit signed copy of the report at least 30 days prior the thesis defense. The Reviewers are asked to bring a copy of the completed report to the thesis defense and to discuss the contents of each report with each other before the thesis defense.

If the reviewers have any queries about the thesis which they wish to raise in advance, please contact the Chair of the Jury.

Reviewer's Report

Reviewers report should contain the following items:

- Brief evaluation of the thesis quality and overall structure of the dissertation.
- The relevance of the topic of dissertation work to its actual content
- The relevance of the methods used in the dissertation
- The scientific significance of the results obtained and their compliance with the international level and current state of the art
- The relevance of the obtained results to applications (if applicable)
- The quality of publications

The summary of issues to be addressed before/during the thesis defense

The thesis focuses on the analysis of the lipid composition of breast milk and brain tissue in a number of mammalian species to investigate evolutionary changes in the milk lipidome and explore the coevolution of the milk and the postnatal baby brain lipidome composition. While previous studies assessed the general lipid composition of milk at the lipid class level, the systematic analysis of individual lipids and their fatty acids conducted using untargeted liquid chromatography coupled to mass spectrometry (LC-MS), as well as the analysis of the relationship between breast milk and growing baby brain lipidome composition, was lacking.

The thesis is well written, presenting a good introduction to the research topic and the methods used in the analysis. In the introduction, the author also formulates her hypothesis that brain lipidome evolution might be critically linked with the breast milk composition in all mammals, including humans, given rapid brain growth in early postnatal development supported by milk as the only source of nutrients.

The first study focused on the test cohort of 38 breast milk samples from seven species representing three phylogenetic groups: primates (humans, rhesus-macaques and crab-eating macaques), bovidae (cow, yak, and goat) and suidae (pig). Lipids in milk are mainly represented by triacylglycerides (TAGs) - glycerolipids composed of three fatty acids bound to a glycerol backbone. The author characterizes TAGs contained in the breast milk of different species by the cumulative chain length of the fatty acid residues and the total number of double bonds. The results demonstrate that breast milk of different species has a unique TAG composition: pig milk contains more polyunsaturated TAGs, cow and goat milk has more short- and medium-chain TAGs with low level of unsaturation, and human breast milk has more medium- and long-chain TAGs with medium to high levels of unsaturation.

In the second part of her research, the author presents an analysis of the main study cohort containing more than 1,000 breast milk samples from the same seven species (humans, monkeys, cows, goats, yak, pigs), as well as approximately 250 brain tissue samples taken from the prefrontal and cerebellar cortices in four of the seven species. In addition to studying the total lipidome, the author performs hydrolysis to cleave fatty acid residues from glycerolipids. Annotated fatty acids show species-specific distribution, with short and saturated ones overrepresented in the breast milk of bovids, very long and unsaturated - in primates, and polyunsaturated - in pigs. The analysis further reveals a group of fatty acids uniquely overrepresented in humans compared to all other species, including the non-human primates.

The same experimental procedure was applied to brain samples. The author shows that the group of long-chain unsaturated fatty acids is overrepresented in primates, and

particularly in humans. Interestingly, the excess of human-specific differences in the fatty acid composition was stronger in the prefrontal cortex compared to the cerebellum. Furthermore, the results demonstrate a positive relationship between fatty acid abundance in breast milk and growing brain tissue during the first several months of postnatal development, thus confirming the author's hypothesis.

In the discussion, the author points towards the importance of the very-long-chain fatty acids in the brain and addresses the controversial hypothesis designating breast milk as the possible source of these fatty acids. However, no studies have yet revealed the direct transfer of the long-chain unsaturated fatty acids from milk to the brain.

Overall, the structure of the thesis meets the requirements. Investigation of lipids by mass spectrometry was performed at the high professional level using modern experimental approaches. However, I have a number of comments to be addressed:

1. On p. 20 of Introduction part 1.3 Mass-spectrometry of lipids, the author states that "the advantage of metabolomics is its lower cost compared to other omics platforms, such as genomics, transcriptomics, and proteomics". Metabolomics is the same cost as proteomics. Please rewrite the phrase.
2. Speaking about the invention of the two techniques, matrix-assisted laser desorption ionization, MALDI, and electrospray ionization, ESI, the author gives erroneous citation (Han and Gross 1994; Kim 1994) on p.21 of the same chapter. Correct citation has to be included.
3. Author writes that "double bond configurations cannot be assigned based on tandem mass-spectrometry" p.22, however there are several mass-spectrometry techniques that allow investigation of the double bonds.
4. The legend of fig. 4 is not clear, specify how the pattern on the right was obtained.
5. p-values in fig. 16 p. 69 need to be rounded.
6. Author uses the identification of lipid species generated by their own annotation method, but it should be taken into account that the method allows to assign particular lipids only with a certain degree of accuracy.

Provisional Recommendation
☒ *I recommend that the candidate should defend the thesis by means of a formal thesis defense*
☐*I recommend that the candidate should defend the thesis by means of a formal thesis defense only after appropriate changes would be introduced in candidate's thesis according to the recommendations of the present report*
☐*The thesis is not acceptable and I recommend that the candidate be exempt from the formal thesis defense*