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Jury Member Report – Doctor of Philosophy thesis.

Name of Candidate:	Vita Stepanova
PhD Program:	Life Sciences
Title of Thesis:	Metabolic variations of modern and ancient human populations
Supervisor:	Prof. Philipp Khaitovich
Date of Thesis Defense:	20 December 2019
Name of the Reviewer:	Vasily Ramensky

I confirm the absence of any conflict of interest	**Signature:**  **Date: 05-12-2019**

Reviewer's Report

The doctoral thesis of Vita Stepanova is devoted to investigations of metabolic variations in human populations. The major result of the study is the description of modern human-specific changes in brain metabolome and lipidome and its inter-population variations.

The thesis is well-written, comprises 90 pages, its composition resembles a paper, with Abstract, Literature Overview, Results, Methods and Discussion sections, followed by Conclusion and Bibliography. Each section contains sub-sections, the overall structure of the text is transparent and logical. The actual content of the thesis represents the topic of the study quite well. One may complain however about somewhat small figure size and brevity of the Overview section.

Chapter 5 gives an overview of the methods used in the project which include mass spectrometry data processing and downstream statistical and bioinformatic analysis. The author conducted most of the bioinformatics analysis presented in the thesis, including statistical analysis and annotation of molecular compounds.

The thesis presents a systematic analysis of the lipidome and metabolome organization of the brain in ~300 individuals from Han Chinese, Western European, and African American populations. This is the first large-scale study reporting the presence of substantial lipid and metabolic brain composition differences among contemporary human populations. This difference is age-dependent, with most differences observed in young Han Chinese individuals, and involves metabolites and lipids clustering in specific metabolic pathways.

The publication list of the author contains 6 papers in high quality international journals with three of them describing the contents of the thesis. The results of the study are novel and of interest to the research community. Below I give my questions and comments.

1) In the section «Statistical analysis of lipid and metabolite differences among populations» the samples were separated into two subsets: DS:0-4 (n = 74, ages less than five years), and DS:5-71 (n = 229, ages from five to 71 years). For this specific type of analysis based on direct comparison of populations, the author shows that the observed specific lipidome behavior of Han Chinese population in DS:5-71 was not due to the difference in statistical power between the age-based subsets. My

question is why the age was not used as one of predictor variables in the subsequent population classification using machine learning. Instead, the classification procedure was applied to the DS:5-71 only.

2) One of the most interesting results of this work is the role of Ala429Val substitution in *ADSL* purine metabolism. When compared to primates, the purine biosynthesis stands out as down-regulated in humans, particularly in the brain. In human cells, the ancestral version of *ADSL* supports a higher level of purine biosynthesis than the present-day, modern human version. It is hypothsized that the Ala429Val substitution does not affect the kinetics of the murine *ADSL* enzyme, but instead destabilizes the secondary structure of the protein. Taking in view that the spatial structure for *ADSL* is available, I feel like the study would clearly benefit from the most basic bioinformatic analysis of the role of the residue on the protein function and structure. In particular, I seem to miss a figure of the protein with designated binding and substitution sites. Another thing is the prediction of the free energy change upon mutation with FoldX or related tool, although the performace of these tools is far from perfect.

3) There are few typos and not very clear passages in the text, e.g.

p.2. *population differences on metabolome and lipidome level*

p.6: *Discovering the metabolic traits of modern individuals I study...*

p.7: *We hypothesized and that the single amino acid substitution...*

p.15: *It results in technical difficulties or at least need to choose between several options of*

metabolic measurement design...

These comments by no means undermine or challenge the value of the study and its presentation in the thesis. My conclusion is that Vita Stepanova, the author of the thesis proved her ability to perform research and to achieve valuable scientific results. Therefore I recommend the thesis for presentation with the aim of receiving the Ph.D. Degree.

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*