Reviewer's Report on Dissertation Thesis

"Pathology and physiology de novo purine synthesis"

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This Dissertation Thesis focuses on pathology and physiology *de novo* purine synthesis. First, the author prepared *de novo* purine synthesis (DNPS) substrates that were used to develop liquid chromatography–tandem mass spectrometry (LC–MS/MS) and liquid chromatography–high-resolution mass spectrometry (LC–HRMS) methods for their detection. Then, the author characterized cell lines as models for individual DNPS defects. Last but not least, the author applied LC–MS-based methods for screening of biomarkers of known and putative DNPS disorders in urine and dry blood spot samples.

The work presented in this Dissertation Thesis is new and original and brings a new light on pathology and physiology *de novo* purine synthesis. The main outcomes presented in the Dissertation Thesis have been published in peer-review journals.

Comments on the Dissertation Thesis:

- This Dissertation Thesis contains many abbreviations. It would be convenient for readers to provide a list of those ten target purine metabolites and their abbreviated forms near Figure 5 (not only in List of abbreviations, pp. 11–13). In fact, Figure 5 is the same as in Appendix II (Baresova V *et al.*, *Molecular Genetics and Metabolism* 119 (2016) 270) in which case also abbreviations are included.
- Appendix III: It would be valuable to deposit high-resolution MS/MS spectra for all target compounds in some open-access repository such as MassBank of North America, MoNA (mona.fiehnlab.ucdavis.edu) so other researchers would have access to them.
- pp. 58–60: Only six purine metabolites were analyzed in urine and dried blood spot samples. What was the reason to exclude the remaining four purine metabolites (out of 10)?

Questions for discussion:

1. The Dissertation Thesis focuses on the targeted analysis of purine metabolites which is basically limited to 10 compounds. Are there any studies that would study DNPS-disrupting disorders also using the global (untargeted) metabolomics approach?
2. Did you compare limits of detection between dried blood spot samples and for instance blood plasma? As shown in Table 7 (p. 60), some purine metabolites were not detected in dried blood spot samples which might be due to a low amount of biological samples used for analysis.
3. Based on your studies and studies conducted elsewhere, are there solid reference values that would allow to conclude which step of *de novo* purine synthesis is impacted (similarly to newborn screening for metabolic disorders based on targeted analysis of acylcarnitines and amino acids)?

Overall, the Dissertation Thesis of Mgr. Matyáš Krijt is of high quality and the author has proven to be creative in study design and implementation of methods used for a particular research topic. Therefore, I recommend accepting this Dissertation Thesis for defense.

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