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Reviewers report – diploma thesis authored by Mônica Jandová

The diploma thesis of Mônica Jandová aims to elucidate the impact of the gluten-free diet on the chemically induced mouse model of Parkinson's disease. This is timely, as the incidence of Parkinson's disease is growing in the population and some studies associated the induction or progression of Parkinson's disease with the changes in the gut microbiome. If taken seriously and performed well, this project might reach important conclusions with even translational potential.

The diploma thesis is written in English and has a standard composition of chapters. The introductory part aims to describe the pathogenesis of Parkinson's disease, the involvement of the immune system in its onset and progression, the possibilities of therapeutic interventions, and the animal models of Parkinson's disease – all on approximately seventeen pages. This leads to a somewhat superficial, descriptive, and shallow description related to individual topics. There is a minimal synthesis of information from various resources, as can be clearly documented by the overuse of secondary (review) citations. Given the topic of the thesis and the study program selected by the applicant, the reviewer would assume that the parts related to animal models of Parkinson's disease and the role of the immune system in Parkinson's disease would be the main sections of the literature overview. Yet, this is absolutely not true. Just for example, the chapter related to Tregs and Parkinson's disease cites only a single work out of numerous published. This is far from being the exclusive, well-set, and extinguishing review of the literature that the introductory part of the thesis is supposed to be. Similarly, numerous parts of the thesis lack the sufficient detail needed for this type of text. The most striking examples can be found below:

> "*Memory T cells may also play a role in the onset and progression of PD, according to mounting data (Dhanwani et al., 2022; Galiano-Landeira et al., 2020; Seledtsov & von Delwig, 2020).*"

> "*Several studies documented possible beneficial effects of dietary interventions in PD (Mischley, Lau, and Bennett 2017; Bisaglia 2022).*"
> There are probably many cell types that can play a role in the onset and progression of Parkinson's disease, but when lacking the mechanism behind it, this has almost zero information value. On the same note – how is the beneficial effect of the diet achieved, what were those papers able to show?

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"*NK cells have the ability to home to the CNS in neurological disorders such as PD…*" How is this possible given the existence of the blood-brain barrier?

"The gut microbiota is composed of crucial bacteria that are responsible for maintaining the integrity of the gut barrier and immune system regulation." – Which bacteria do so and how do they achieve this?

"PD patients appear to have had a lower abundance of bacteria from the Lachnospiraceae family and a higher abundance of bacteria from the Verrucomicrobiaceae family compared to healthy controls (Scheperjans et al., 2015). These alterations in the gut microbiota have been connected to the buildup of the alpha-synuclein protein in the gastrointestinal tract. Alpha-synuclein is thought to assemble in the gut as a result of gut dysbiosis, which may then spread to the brain via the vagus nerve and enhance neurodegeneration in PD." – Once again, how is this supposed to work mechanistically?

Secondly, a substantial part of the citations used in the introductory part is, for some reason, lacking in the reference list (e.g., Lei et al., 2021, Ho et al., 2019, Affifi et al., 2017 to mention just a few). This makes the reviewing of the thesis almost impossible as it is not clear which particular study the citation is referring to.

Third, sometimes in the literature overview, the data obtained from animal models of Parkinson's disease and patients suffering from it are not clearly separated. It is thus not clear from which condition this data were obtained, and too dangerous generalization occurs.

In summary, the literature overview is far from standards imposed on the scientific text as well as on the diploma thesis of the Immunology program and needs complex and deep rewriting in order to match current standards.
The aims of the experimental part are well-stated and interesting. They consist of the establishment of a chemically induced Parkinson's disease mouse model in the laboratory and subjecting those mice to a standard or gluten-free diet. Various immunological parameters are then supposed to be measured in those mice. Lastly, the extraction of PBMCs from the blood of Parkinson's disease patients under different dietary regimes was supposed to be done in order to measure some immunological parameters. The part devoted to Parkinson's disease-bearing patients is described in Aims and Methods sections (without any data regarding the cohort of patients), but there is no data devoted to these experiments in the Results section. It is not clear to me how this can happen and that this apparent issue was not recognized during the review of the thesis in the laboratory.

The experimental part starts with the establishment of the mouse model of chemically induced Parkinson's disease. It is not really clear what is taken as the quantitative benchmark for the successful establishment of this model.

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The only readout seems to be the immunofluorescence of (probably – not clearly specified in the thesis on pg. 38) the anti-hydroxylase staining that was performed on the brain tissue sections. This histology should be presented with an additional marker, to assure that the same part of the tissue is visualized in both conditions. The scale bar is missing, and it is not stressed if this essential control was performed for all the animals. The same applies to Figure 9 on page 39.

Then the thesis continues with the open field tests of the behavior of mice subjected to chemically induced Parkinson's disease and fed by the standard or gluten-free diet. There are NO statistically significant changes in any of the parameters measured (Figure 10, pg. 40). Yet, to the great surprise, this is interpreted in the discussion and conclusions as the striking outcome of the study (e.g., page 61 *"The results revealed a significantly higher exploration value among mice on the GFD compared to those on the STD. This higher exploration value indicates an increased willingness of GFD mice to explore and engage with their environment."*). This is not acceptable and in absolute contrast to the obtained data. There are more issues associated with this experiment - the cohort without the induction of Parkinson's disease-like conditions is lacking, thus there is not a single test-taking to account solely for the effect of the standard and gluten-free diet on the above-mentioned parameters. Also, it is not specified if the experimental cohort are littermates and when they were started to be fed by standard and gluten-free diet.

The last and most dense part of the diploma thesis is devoted to measuring the changes in cellular immune compositions in above mentioned experimental animals. It is not clear to me why if the authors are interested in how gut-imposed changes translate to the CNS the immune parameters were measured only systemically and not also in the gut and CNS. More specifically to this dataset: it is impossible to evaluate the general gating strategy on page 42 (Figure 12) due to the super-low resolution of the figure. In all the remaining figures, the number of experiment repetitions and animals used is missing. No figures are documenting the changes in particular populations between standard and gluten-free diet conditions for any of the graphs presented. Particularly, I would like to see the Foxp3 staining in CD8+ T-cells and gamma-delta TCR-bearing cells. Lastly, some FMOs are provided, yet probably the most necessary one for NKg2d PE is missing.

The last part of the thesis is devoted to the discussion of the obtained data. This part serves more as a summary of the results section than the discussion, Given the number of datasets available from the chemically induced Parkinson's disease model, I would anticipate a deeper discussion of obtained data in comparison to other studies. The exaggerations in the discussion and conclusions were already mentioned. Reporting the negative data in the diploma thesis is absolutely fine, but they need to be discussed properly. This is unfortunately not happening in this case.

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In summary, the diploma thesis of Mônica Jandová is problematic in so many aspects. The introductory section and discussion are written in a shallow manner, lacking the synthesis of the information from various sources and the mechanistic detail. The experimental part lacks essential controls, data are not reported in proper way and the conclusions are based on overinterpretations not aligning to the data provided. Multiple mistakes violate the correct style of the diploma thesis – e.g. missing citations, missing part of the results (or unrelated part of the text in methods and aim sections if you wish), incomplete figure legends, etc. Thus, in the current form, the diploma thesis does not match the criteria of the Immunology study program, Unfortunately, I cannot recommend this thesis for the defense in current form.

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