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3rd January, 2022

Review on a doctoral thesis of Katarzyna I. Szczerkowska titled "Function of Zinc finger protein 644 (Zfp644) in mouse organism".

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Co-Supervisor: Jan Prochazka, PhD
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As the thesis was written in English the following report retains the same language.

The thesis is divided into standard chapters, such as Introduction, Aims of the Study, Material and methods, Results and Discussion. The main body precedes an abstract in both English and Czech languages and the work is concluded by a short summary. Publications, abbreviations, Figures and tables are listed at the end of the theses.

The theses presents a novel data on ZNF644, WIZ and G9a/GLP complex and its molecular mechanism and points a similarity between human and mouse Zinc Finger Protein 644 which could be used in follow-up targeted studies towards understanding of human myopia. The applicant is well aware that further research on the G9a/GLP methylation complex and its action in the organism, influenced by ZNF644 and WIZ is necessary.

I) The Introduction describes the scientific background of the studied subject and reflects on the current literature. An order of the introductory chapters it is probably matter of preference. In my opinion, I would at first, at least briefly, introduced Myopia, as it is the key targeted disease for e.g. aim 1) and combined it with the Zfp644 knowledge to create cohesive story. On the other hand, I value, that the figures are relevant and they nicely complement the text. It is pity that that figure legends are not unified in formatting (either centre or left, not either or).

II) The aims of the study are well defined into three parts:

1) Clarify the role of Zfp644 in the development of myopia disease in vivo using murine models. Within this aim, 2 mouse models of Zfp644 protein were created to prove that Zfp644 is causative for developing high myopia.

2) Reveal the physiological role and a molecular function of Zfp644 in mouse organism and explain the role of the transcription factor in female reproduction. Within this aim the interaction pathway between Zfp644 and G9a in female fertility in cell lines and murine models *in vivo*.

3) By complementary project to describe a role of Wiz in G9a/GLP complex. Within this aim the role of Zfp644 was complemented by Wiz impact on murine development.

III. Materials and methods are well described, and I can imagine that experimental part could be repeated by reading them also published material is being used. I am not only happy about the lack of clarification between genes, proteins, GMO models, please see my Comment C2.

IV. Results are presented in subchapters in logical order, accompanied by representative Figures and legends. Certain discrepancies are commented again in C2. Also, certain figures are not well addressed, particularly Figure 4.7. see Comment C3, Question Q3. Also Tables 1 a 2 are very small and could have been placed on individual page lengthwise, same for e.g. Figure 4.18 and many others.
In general, the results presented are of larger scale and deliver the knowledge of the applicant in the topic, using wide range of approaches. Among many, it is impressive the finding that Wiz deficiency leads to developmental defects especially in craniofacial area, growth retardation, and embryonic sub-viability using micro-CT images of mouse embryos and analysis of H3KO methylation pattern towards a role of Wiz and G9a/GLP in palate development, sadly leaving rather open ending story.

V. Discussion shows a good attempt to bring the whole story together and points on unique and very useful new transgenic models compares to previously available. It is pity that the comparison is not well addressed and for example the limitations are listed as references 97, 140, 98, 99, 100 and 101 (and as you can see not totally in numerical order, which is again one of downside of the whole thesis). The reader would also probably appreciate discussion as such based on obtained results when lack of results or lack of antibodies etc could be all included in one part discussing the trouble shouting and ideal future aspects. On the other hand among many other positives the potential molecular mechanisms in Zfp644 in developed transgenic model (Figure 6.1.) nicely illustrates applicant in dept knowledge, even though the Figure should have been numbered 5.1.

VI. Summary reflects on Aims and summarise the deliveries such as:

1) Based on single point mutation selected from GWAS studies, the mutations of Zfp644 are causative of myopia phenotype in mice.
2) Molecular mechanism behind the observed phenotype needs further studies, however, the physiological role of Zfp644 on a mouse organism and female subfertility phenotype in *Zfp644* $^{\Delta 8}$ animals were described.
3) The function of Wiz on craniofacial development as well as on methylation pattern of H3K9 in the developing mouse craniofacial area was described.

In my opinion, the summary of aims 2 and 3 could have been clearly stated in a summary sentence saying what was actually “described”.

To summaries this review, the thesis present quality data published in two publications, where the applicant is the first author (Cell Biosci. 2019 Feb 21;9:21.) and second author (Front. Cell Dev. Biol. 2021, 9:620692).
The thesis "Function of Zinc finger protein 644 (Zfp644) in mouse organism" of Katarzyna Szczerkowska is scientifically sound and the above critical points do not decrease the quality of scientific work presented. I recommend this work to be considered for obtaining a doctoral degree by respectful members of doctoral board committee.

Yours sincerely,

Katerina Komrsková

Comments (C) / Questions (Q)

C1: Aims of the Study pg. 28.
It would be useful to define briefly within this chapter the role of WIZ (Wiz) in the G9a/GLP methylation complex, and why it was interesting to look into impact of Wiz on murine development. The Aims should be self-explanatory even without the Introduction and the WIZ (Wiz) part is neglected.

Q1: Could applicant define as an extension of "Aims of the study" the reasons of merging the Wiz and Zfp644 story together?

C2: It is not clear to me, why using correctly italics for genes (abstract, majority of Introduction) had not been remained withing the whole theses, where referred to genes or GMO mouse models, particularly it is omitted in Material and methods, some in Results (especially some figure legends). Italics was even not used in Aim 1. for "*in vivo*", in one case. These and truly many other discrepancies should have been taken care of.

Q2: Could applicant make a comment?

C3: Figure 4.7. (A) legend does not explain the band of 100kDa. (B) refers to non-significant differences in upregulation for lepr and its isoforms. Not only that the figure legend is not well described with mistakes, but the error bars are not inserted.

Q3: Could applicant address the figure 4.7. A, B and add the missing information?

Q4: How would applicant design follow-up study to address connection between epigenetic chromatin modification and craniofacial defects?

Q5: The ending of Discussion is very bleak stating: "Hopefully, the experiments that are being performed simultaneously with the thesis writing would bring us closer to revealing the role of Zinc Finger 644 in G9a/GLP complex".
Please explore and state clearly point by point, what experiments are needed to reveal the role of Zinc Finger 644 in G9a/GLP complex

Q6: Could applicant clearly state in Summary pg. 95 for Aims 2 and 3, what was described?