**Doctoral Dissertation Evaluation Report**

**Candidate: Ms. Mina Rajkovic**

**Dissertation title: Functional significance of DHX38 in pre-mRNA splicing and its role in the mechanism of retinitis pigmentosa.**

Although objectives of the submitted doctoral thesis are not explicitly delineated, the work is well focused on the role of pre-mRNA splicing in retinitis pigmentosa. The thesis is based on candidate's first-authored publication: Obuća M., Cvačková Z., Kubovčiak J., Kolář M. and Staněk D., 2022, Retinitis pigmentosa-linked mutation in DHX38 modulates its splicing activity. PLoS One. 2022, Apr 6;17(4): e0265742. doi: 10.1371/journal.pone.0265742. Besides that, it contains a minor loosely associated result from the publication Královicová J, Ševčíková I, Stejskalová E, Obuca M, Hiller M, Stanek D, Vorechovský I. (2018) PUF60-activated exons uncover altered 3' splice-site selection by germline missense mutations in a single RRM. Nucleic Acids Res. 2018 Jul 6;46(12):6166-6187. doi: 10.1093/nar/gky389, which was also co-authored by the candidate. Relative long time from the publication of both manuscripts allowed them to prove their significant value for the scientific community. In total, these publications have been cited 39 times according to Web of Science and 50 times according to Google Scholar.

The dissertation is organized in a traditional manner, comprising chapters Abstract, Literature overview, Material and methods, Results, Discussion, Summary, References and Supplementary material. The literature overview is well written, although it is based rather on older publications and substantial number of reviews.

I have following questions and comments:

I had to use a magnifying glass to be able to read descriptions in Figure 19 D/E. It appears that there is not much agreement between RT-qPCR and RNA-Seq data. Could author comment these results in more detail?

I understand that cycloheximide has been often used as an NMD inhibitor. However, this compound is a very potent inhibitor of translation elongation and consequently a potent inhibitor of almost all processes in the cell. I would not recommend using it in such a kind of prospecting research because it can elicit many off-target effects. Could author suggest—in light of the current developments in cellular and molecular biology—some possible better methods leading to more specific NMD inhibition?

Could author comment in more detail how the bioinformatical analysis of the degree of splicing was performed, in particular, how genes with better spliced introns were recognized and filtered out?

I must mention that I am personally not a big fan of using HEK293 cells. Please, could you comment on why did you chose this cell line and summarize pros and cons, if any, for using this cellular model in the alternative splicing studies?

Author shows that GFP-tagged DHX38 doesn't interact with the SNRNP200/BRR2 protein complex (Figure 13). In another experiment, SNRNP200/BRR2 demonstrates the highest enrichment among all the proteins tested in the DHX38-FLAG pull-down assays. Could author show both these constructs

and comment this discrepancy in more detail? The role of SNRNP200/BRR2 in splicing and its possible involvement in some variants of retinitis pigmentosa (RP) is well described in the thesis. However, possible joint role of SNRNP200/BRR2 and DHX38 in splicing and RP development is discussed only marginally. Could author try to hypothesize more on this?

One of the most surprising results is no association of DHX38 with snRNAs. Is there any possibility how the pull-down assay could be modified to increase both the sensitivity of the snRNA detection and probability that the DHX-containing complexes will remain undissociated and undamaged during the procedure?

In some cases, the scientific work delivers exciting results almost immediately. In many cases this is not true and the chosen experimental model resists to most of our attempts to get more inside the understanding of its mode of function. DHX38 seems to be rather the latter case, and I guess that Mina Rajkovic spent a lot of time to get at least "something" from it. However, scientists have to take risks and have to attempt these kinds of studies knowing that straight ways often do not go to the goal and sometimes the main goal even remains unachieved. I am very sure that Mina Rajkovic has learnt a lot during her doctoral studies and has become ready for the independent scientific life.

To summarize all that, the submitted dissertation contains original results and demonstrates, in my opinion, ability and readiness of Ms. Mina Rajkovic for the independent research. It is thus my kind obligation to conclude that the thesis and the publications on which it is based meet as a whole all the professional and formal requirements for a doctoral dissertation and Ms. Mina Rajkovic has thus fulfilled all the requirements demanded by §47, article 4 of Act on Higher Education Institutions No. 111/1998. Based on that, I recommend the thesis to be accepted for defence and as a basis for the Ph.D. degree award.

RNDr. Martin Pospíšek, Ph.D.
Department of Genetics and Microbiology
Faculty of Science, Charles University
Viničná 5, 120 00 Praha 2
Czech Republic