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CENTRE de RECHERCHE en MYOLOGIE

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Office for Organizational Support of
Dissertation Councils, Administration
Saint Petersburg State University

Paris May 1st, 2023

Please find enclosed my review of the dissertation of Ms Perepelina Kseniia Igoevna.

Yours Sincerely,

Gisèle Bonne
DR Inserm

РК № 33-06-541 от 03.05.2023

REVIEW

Of the member of the dissertation council for the dissertation of ***Perepelina Kseniia Igoevna*** on the topic: "***Impact of the tissue-specific mutations in the LMNA gene on cell differentiation***", submitted for the degree of ***Candidate of Biological Sciences*** in a scientific speciality 1.5.22 Cell Biology

Ms Perepelina Kseniia Igoevna is presenting a dissertation manuscript for a defence on May 15th, 2023.

This thesis is a synthesis of work carried out in the field of cell biology aiming at the understanding the pathomechanisms of *LMNA* gene mutations leading to a wide spectrum of rare genetic diseases, the laminopathies. Ms Perepelina thesis work focused on the study of the impact of various mutations in the *LMNA* gene on the processes of cell differentiation.

This work, carried out under the supervision of Dr Malashicheva Anna Borisovna has been productive and led to the publication of 5 original articles with Ms Kseniia Perepelina as first author in *Cytology* (2017), *Biochem. Cell Biol* (2018), *Cells* (2019), *Stem Cell Res* (2020) and *Front. Cardiovasc. Med.* (2022) and one review article as second author in *Front. Cell Dev. Biol.* (2021). She has also presented her work at different international congress with 3 published abstracts.

The thesis manuscript of Ms Kseniia Perepelina is built according to a classical scheme of literature review, experimental methods, results and discussion.

The literature review is well developed and recalls in a relatively complete way the knowledge necessary for someone coming from another field to understand the thesis work. An effort has been made to enable the reader to grasp the complexity of the known roles and functions of lamins A and C as well as the wide variety of laminopathies.

The chapter "Materials and Methods" presents the various cell models used as well as the cell biology, molecular biology and imaging techniques used in this work.

The results are presented in 4 chapters and correspond to the exploration of 4 different studied cell differentiation models: 1) mouse C2C12 myoblasts and mouse primary muscle satellite cells for muscle differentiation, 2) human cardiac mesenchymal cells and their adapted differentiation protocol for adipogenic differentiation, 3) different types of human cells of mesenchymal origin (umbilical vein endothelial cells, cardiac mesenchymal cells, aortic valve smooth muscle cells and aortic valve interstitial cells) to explore osteogenic differentiation and finally 4) induced pluripotent stem cells (iPSCs) derived from laminopathic patient and differentiated in cardiomyocytes.

Mutations in the *LMNA* gene encoding A-type Lamin (i.e. lamin A/C) give rise to a variety of genetic diseases that are either tissue specific such as a range of muscle and cardiac dystrophies (Emery-Dreifuss muscular dystrophy (EDMD), Limb-girdle muscular dystrophy (LGMD1B), *LMNA* related congenital muscular dystrophy (L-CMD) and isolated dilated cardiomyopathy with conduction defects (DCM-CD)) affecting the striated muscles, partial lipodystrophy (FPLD) affecting the adipose tissue, axonal neuropathies (CMT2B1) affecting the peripheral nerves, or more systemic syndromes with a range of premature ageing syndromes (Hutchinson-Gilford progeria (HGPS), mandibulo-acral dysplasia (MAD)…). The phenotype-genotype relations are far from being clear, as the pathomechanisms tat lead from the gene defects to the specific diseases. Therefore, the work of Ms Ms Kseniia Perepelina focuses on the analyse of specific *LMNA* associated with tissue specific diseases and their impact on the related tissue differentiation.

She thus, explored the impact on myogenic differentiation of the G232E and R571S mutation that were reported in patients presenting with respectively EDMD and LGMD and revealed myogenic differentiation defects in mouse primary satellite cells and C2C12 cells (*Cytology*, 2017).
Similarly, she explored the R482L mutations reported in patients with FPLD and observed abnormal adipogenic differentiation together with a decrease in the activity of the Notch signaling pathway in human cardiac mesenchymal cells under adipogenic differentiation protocol (*Biochem. Cell Biol*, 2018). She further explored the impact of the R527C and R471C mutations reported in patients presenting with MAD, on the osteogenic differentiation in different types of human cells of mesenchymal origin. She observed opposite effect of the R527C mutation on Notch signaling activity and osteogenic differentiation in human heart mesenchymal cells and human aortic valve interstitial cells (*Cells*, 2019). She finally, derived cardiomyocytes form induced pluripotent stem cells (IPSC) of a patient presenting with EDMD associated with the R249Q mutation (*Stem Cell Res*, 2020) and explored their cardiogenic differentiation capacity and revealed altered electrophysiological properties of the cardiac sodium channel compared to cardiomyocytes obtained from IPSC from healthy donor (*Front. Cardiovasc. Med.*, 2022).

In the discussion sections of the dissertation, the results obtained are not over-interpreted and are analyzed in a lucid manner defining the limitations of the results as well as the experiments needed to complement and reinforce these results. It is true that one would have liked to have had the exploration of the same *LMNA* mutations in all the different cells models to reinforce the results and their tissue specific roles and impacts. But this would have most probably necessitated a much larger amount of work to be developed.

During the course of her work, Ms. Kseniia Perepelina implemented various cell biology and molecular biology techniques on various cell models. The approaches discussed indicate that Ms. Kseniia Perepelina has found her way into the complex field of cell models and has been able to make use of them.

Many interesting points of the thesis work will be discussed during the oral defence. Possible extensions of the work in the short and medium term will be discussed.

In conclusion, I'm in favour of the defence of Ms Kseniia Perepelina for obtaining the grade of *Candidate of Biological sciences.*

Dissertation of *Perepelina Kseniia Igoevna* on the topic: "*Impact of the tissue-specific mutations in the LMNA gene on cell differentiation*" meets the basic requirements established by Order No.11181/1 dd. 19.11.2021 "On the procedure for awarding academic degrees at St. Petersburg State University". The applicant *Perepelina Kseniia Igoevna* deserves to be awarded the academic degree of Candidate of Biological sciences in a scientific speciality 1.5.22 Cell Biology

No violations of paragraphs 9 and 11 of the specified Order have been detected.

Member of the Dissertation Council

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Date: May 1st, 2023