

Opinion

Author: Associate Professor Georgi N. Chaldakov, PhD, Department of Translational Stem Cell Biology, Research Institute, Medical University, Varna, on a dissertation for the award of academic degree "Doctor"/PhD in higher education 7. Health and Sports, professional field 7.1. Medicine, doctoral programme in Anatomy, Histology and Cytology.

Applicant: Radoslav Hristov Spasov, MD, Department of Anatomy and Cell Biology, Faculty of Medicine, Medical University - Varna

Topic: *Role of transcription factor Pax6 in mouse cerebellar development*

Scientific supervisor:

Prof. Dr. Anton B. Tonchev, MD, Department of Anatomy and Cell Biology, Medical Faculty, Medical University, Varna

1. Subject of the opinion

By Order No P-109-182/08.04.2025 of the Rector of the Medical University "Prof. Dr. Paraskev Stoyanov", Varna, I was appointed as a member of the Scientific Jury for the provision of a procedure for the defence of a dissertation on the topic "*Role of transcription factor Pax6 in the mouse cerebellar development*" for the acquisition of the educational and scientific degree "Doctor"/PhD in the field of higher education 7. Health and Sports, professional field 7.1. Medicine, Doctoral programme in Anatomy, Histology and Cytology. The author of the dissertation is Radoslav Hristov Spasov, Department of Anatomy and Cell Biology, Faculty of Medicine, Medical University 'Prof. Dr. Paraskev Stoyanov', Varna.

The presented set of materials **is in accordance** with the Procedure for the Acquisition of an Educational and Scientific Degree (PhD) at the Medical University of Varna (MUV) and includes all the necessary documents in accordance with the Academic Staff Development Act (ZRAS) in the Republic of Bulgaria and the MU-Varna Rules for Admission to the PhD Defense.

2. General data on the dissertation

The dissertation is presented on 139 pages, including 124 cited sources, 55 figures, 33 diagrams and 3 tables – these, as well as the structure of the thesis, are in line with the norms for the award of the degree "Doctor"/PhD.

3. Short biographical data about the dissertation

Radoslav Spasov was born in Kyustendil in 1988. He graduated in Medicine in 2014. Since September 2015 he has been a part-time assistant professor at the Department of Anatomy and Cell Biology at MU-Varna. In March 2016 he was appointed as a regular assistant at the Department. He joined the doctoral programme at MU-Varna in January 2018. In May 2021, after passing an examination, he specialised in Anatomy, Histology and Cytology. Dr. Spasov's research interests are focused on cellular and molecular neurobiology. He is a member of the Bulgarian Anatomical Society and the Bulgarian Medical Association. In 2022, he was awarded as the *favorite lecturer* of the graduating course in Bulgarian-language medical training at MU-Varna.

4. Relevance of the dissertation and of the objectives and tasks set

The cerebellum contains most of the neurons in the central nervous system. In addition to the 'classical' involvement in motor control, modern data also show its role in cognitive processes, as well as in disorders of higher nervous activities such as *autism spectrum disorders* and other disorders of small brain development. Dysfunction of *pax6/PAX6* is involved in the pathogenesis of glioblastoma. The proliferation of granular cerebellar neurons is a critical pathogenetic mechanism for the development of the most common brain tumour in children, medulloblastoma. All this, as well as other data on the pathobiology of *PAX6*, are important prerequisites for investigating the cellular and genetic mechanisms of cerebellar neurogenesis in mice.

5. Knowledge of the dissertation topic

The literature review, presented on 21 pages, as well as the cited 124 bibliographic sources, show contemporary awareness of the available literary sources on the dissertation topic. Dr. Spasov has convincingly formulated why he studies the role of the transcription factor paired box6 (*PAX6*), the *gene Pax6* and the transgenic line *Tel-Cre::Pax6^{fl/fl}* in his dissertation. However, it would be desirable to have more citations from the years 2020-2025, for example: Ochi S, Manabe S, Kikkawa T, Osumi N. Thirty years' history since the discovery of *Pax6*: From central nervous system development to neurodevelopmental disorders. *Int J Mol Sci* 2022;23(11):6115. DOI: 10.3390/ijms23116115

6. Methodology of the study

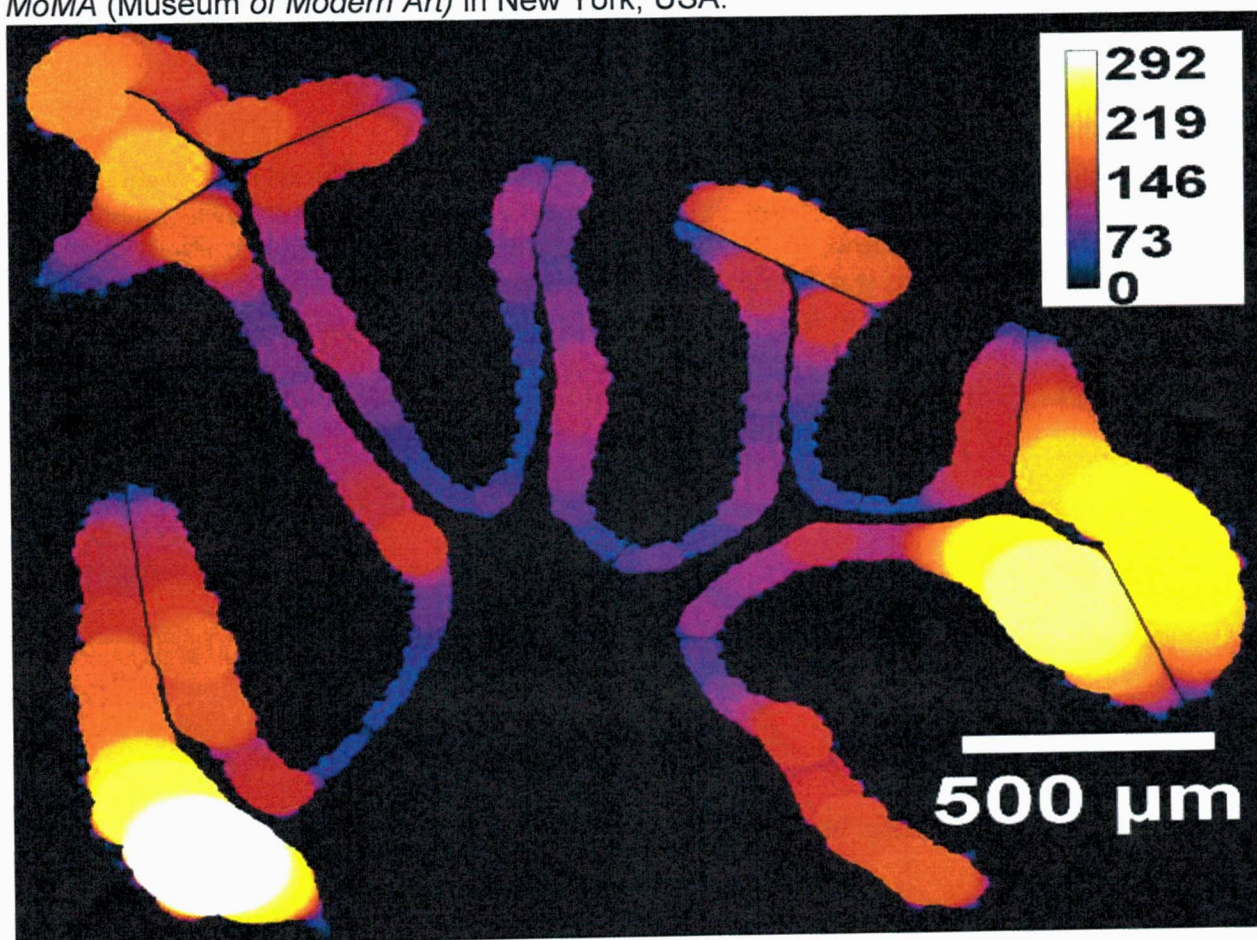
For the dissertation were used tissues from experimental animals created and bred in Germany after permission from a local ethics committee in the state of Lower Saxony, observing the principles of minimizing animal suffering. The *in vivo* gene modification technology in experimental animals known as *Cre/LoxP* was used, in which the enzyme Cre recombinase placed behind a regulatory element of the *Pax6* gene so as to ensure tissue selectivity of expression within the cerebellum, recognizes specific (flox) sequences around the target gene (*Pax6*). In this way, a specific 'knockout' of the gene in the cerebellum is carried out, allowing the animals to overcome the perinatal lethality characteristic for the *Pax6* global knockout. Overcoming perinatal lethality is a great achievement, since the formation of most small-brain neurons in the mouse occurs in the first 3 postnatal weeks. Therefore, the *Pax6* global knockout, which is lethal at birth, is not applicable to the study of granular cerebellar neurogenesis. Successfully achieving *Pax6*

inactivation in the small cerebral cortex allows *the first in vivo results of the effect of reduced Pax6 expression in the cerebellum* to be observed.

The Materials and Methods Section presents in detail histological (histochemical and immunohistochemical) methods of processing, visualization of samples and statistical methods of analysis. The materials and methods are described in a way that allows experiments to be repeated by independent researchers.

7. Characteristics and assessment of the dissertation

The results span 70 standard pages, which contain 2 tables and 55 figures. Many of them are multi-panel, so the total number of figures is significantly higher than 55 – all of them are of very good quality and clearly illustrate the concept of a dissertation. Dr Spasov pursued the purpose and objectives of his dissertation methodically and identified structural changes in the cerebellum of a mouse resulting from selectively reduced expression of the *Pax6* gene. Thus, **for the first time in the world**, Dr Spasov shows the changes in the structure of the cerebellum in these ‘knocked out’ mice, which remain viable and accessible for measuring the area of the individual cortical layers, which has been found to reduce it in the knocked out vs. control mice. The results of Dr. Spasov's enormous work support the scientific meaning of his dissertations. They are high-value and very beautiful – see, for example, this “picture” that can be shown at the prestigious MoMA (Museum of Modern Art) in New York, USA:



8. Contributions and significance of the dissertation for science and practice

This dissertation is an innovative study of the role of Pax6 in postnatal neurogenesis in the cerebellum. Since most cerebellum neurons are formed after birth, it has not been possible to identify changes in the cerebellum with reduced *Pax6* gene expression, because mice with a global *Pax6* knockout die at birth. The thesis describes *for the first time in the world* the processes of reduced expression of Pax6 that occur after the birth in the brain of experimental animals. So the contributions – eight “first time in the world” – fully meet the requirements for acquiring the degree “Doctor”/PhD.

9. Assessment of the publications on the dissertation

The list of publications attached to the procedure for the acquisition of NSA Doctor includes three publications and one participation in a scientific forum on the topic. The dissertator *should* prepare publications of his/her results in journals indexed in *Web of Science*, *Scopus Elsevier* and/or other authoritative bibliographic platforms. To make visible globally another Bulgarian contribution to biomedical science.

10. Autoreferate

And the second abstract, presented together with the dissertation, is made according to there requirements and correctly reflects the main results achieved in the dissertation.

11. Conclusion

The dissertation of Dr. Radoslav Hristov Spasov from the Department of Anatomy and Cell Biology, MU-Varna is dedicated to very interesting neurobiological topic with translational potential. The applicant presents innovative results obtained in a study of *Pax6* knocked out mice. I am confident that the dissertation fully complies with the ZRAS of the Republic of Bulgaria and the Rules of the Medical University of Varna for the acquisition of the degree “Doctor”PhD. I shall therefore vote in favour and allow myself to recommend the same to the other members of the honourable Scientific Jury designated by Order No P-109-182/08.04.2025 of the Rector of the Medical University ‘Prof. Dr. Paraskev Stoyanov’, Varna.

11 May 2025, Varna

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Associate Professor Dr. Georgi N. Chaldakov