REVIEW

From

Prof. Yvetta Antonova Koeva, MD, PhD

Medical University, Plovdiv, Department of Anatomy, Histology, and Embryology of a dissertation for the award of the educational and scientific degree "Doctor"in the field of higher education 7. Healthcare and Sports, professional direction 7.1. Medicine, scientific specialty: "Anatomy, Histology, and Cytology" Code: 03.01.02

Author: Iskren Boyanov Velikov, DMForm of PhD: independent preparationDepartment: Anatomy and Cell Biology at the Medical University of Varna

Topic: "Role of the transcription factor Zbtb20 in the development of the cerebellum"
Scientific supervisor: Prof. Irina Ivanova Stoyanova-van Der Laan, MD, PhD Medical University, Varna, Department of Anatomy and Cell Biology

1. General presentation of the procedure and the doctoral candidate

By Order No. P-109-222/ 28.04.2025 of the Rector of the Medical University - Varna, I have been appointed as a member of the Scientific Jury for the defense procedure of the dissertation titled "Role of the transcription factor Zbtb20 in the development of the cerebellum" for obtaining an educational and scientific degree of "Doctor" in the field of higher education 7. Health care and sports, professional field 7.1. Medicine, doctoral program "Anatomy, histology and cytology". The author of the dissertation is Dr. Iskren Boyanov Velikov from the Department of Anatomy and Cell Biology, MU-Varna.

Dr. Iskren Velikov graduated from the Master's program in Dental Medicine at MU-Plovdiv in 2007. From 2007 to 2008, he worked as a 'Doctor of Dental Medicine' at the Rural Health Service in Razgrad Province. From December 2008 to September 2009, he worked as an assistant in the Department of 'Anatomy, Histology, and Embryology' at MU - Plovdiv. Since 2009 to the present, he has been an assistant in the Department of 'Anatomy and Cell Biology' at the 'Prof. Dr. Paraskev Stoyanov' Medical University - Varna. In 2013, he obtained a specialization in 'Anatomy, Histology, and Cytology'. Since February 2019, he has been a full-time PhD student in the Department of 'Anatomy and Cell Biology'. In February 2024, he was expelled as a regular doctoral student with the right to defend. He teaches macroscopic and microscopic anatomy to Bulgarian and English-speaking students in the fields of Medicine, Dental Medicine, and Pharmacy. He is a member of the Bulgarian Anatomical Society.

The set of materials presented by Dr. Iskren Velikov in paper/electronic format is fully compliant with Article 69 of Section III. PhD candidates withdrawal; Regulations for the development of the academic staff at MU-Varna from December 20, 2019, and includes the following documents:

- 1. Dissertation
- 2. Summary
- 3. Application to the Rector for the initiation of the defense procedure
- 4. Curriculum vitae
- 5. Copy of the master's degree diploma
- 6. Order for enrollment
- 7. Protocol from the doctoral minimum exam

8. Protocol from the department council with a positive decision for readiness for defense

- 9. Order for dismissal with the right to defend
- 10. Declaration of originality
- 11. List of publications related to the topic of the dissertation work
- 12. Declaration of authenticity of the submitted documents
- 13. Declaration for registration of profiles in scientific databases
- 14. Similarity report from the similarity check program of the Publishing Activities Department
 - 15. Report on the existence of an active profile in Google Scholar and ORCID

I have no notes or comments on the documents. The submitted set of documents fully meets the requirements of the Law on the Development of Academic Staff in the Republic of Bulgaria and the Regulations of MU-Varna for admission to the defense of the academic degree 'Doctor'. The doctoral student has submitted **1** publication and a list of **6** participations in national and international scientific forums related to the topic of the dissertation.

2. Relevance of the Topic

The processes of neuronal proliferation, migration, and maturation are under the control of a number of genes, some of which have been the subject of intensive research in recent years. This study focuses on the transcription factor Zbtb20, a new and still insufficiently studied regulator of the neurogenesis of GABAergic neurons, using a model of transgenic mice with Zbtb20 gene inactivation.

The study of the control of neurogenesis, respectively the role of key factors that may be disrupted, is a promising approach for understanding the pathogenesis of a wide range of neurological diseases and would contribute to the development and advancement of clinical neurology. In this regard, the topic chosen by Dr. Iskren Velikov for his dissertation is relevant and of high scientific value. The aim is to formulate adequately in relation to the materials included in the dissertation for research, and the tasks are also fully and accurately defined.

3. Understanding of the problem

The bibliography of the dissertation includes **254** literary sources. It is evident that the dissertation candidate has a good understanding of the problem.

4. Research methodology

The tissue material included in the study is from the cerebellum of mice - normal animals and Zbtb20 mutants (provided in collaboration with the Institute of Microbiology, BAS and Max Planck Institute, Göttingen) at various stages of postnatal development.

A morphometric and immunofluorescent analysis has been conducted (at the Department of Anatomy and Cell Biology, MU-Varna) which includes: measuring the area of the layers of the cerebellar cortex in control mice and mutants of different age periods; quantitative and qualitative assessment of neuronal and neuroglial cell populations in the cerebellum in controls and mutants through the application of a panel of primary antibodies (GFAP, Calbindin, Neun+, Calretinin+, Tbr2+, Ki-67); establishing the expression of Zbtb20 in transgenic mice compared to controls; analysis of images to determine the phenotypic expression and quantitative distribution of cells labeled with specific antibodies (using FIJI/ImageJ software); application of appropriate statistical models for data processing.

Overall, the included study material, the applied methods, the quantitative and phenotypic microscopic analysis of cell populations, as well as the statistical processing of the obtained data fully correspond to the set goals and tasks and have high informative value.

5. Characteristics and evaluation of the dissertation work and its contributions

The dissertation contains **146** standard pages, illustrated with **97** figures of exceptionally high quality. The scientific work has a sufficiently extensive literature review (**30** pages), clearly defined objectives and tasks, and adequately detailed description of the material and methods used.

The sections related to the results and their discussion comprise a total of **78** pages and are presented in a concise and comprehensible scientific style. The conclusions and contributions of the research are clearly formulated and defined. The structure and volume of the dissertation work are in accordance with the requirements for awarding the academic degree of "Doctor".

The original contributions of the dissertation work could be classified as having a *fundamental-theoretical character* - for the first time, the area of the cerebellum and the individual layers of the cerebellar cortex are measured in Zbtb20 mutant mice, as well as the quantitative and phenotypic characterization of neuronal and neuroglial cell populations in the cerebellum of Zbtb20 transgenic mice at different age periods.

6. Assessment of publications and the personal contribution of the doctoral student

The doctoral candidate has presented 1 independent publication related to the topic of the scientific research, as well as a list of **6** participations in national and international scientific forums. The publication is in a Bulgarian journal, Trakia Journal of Science, 2023.

From the review of the presented materials, I could conclude that the conducted scientific research is the result of the doctoral student's personal involvement, and the obtained results and formulated conclusions and contributions are entirely to their personal credit.

The results of this study provide new information regarding the morphogenesis of the cerebellum in mammals and the role of the transcription factor Zbtb20 in this process. Clarifying the genes and their significance in neurogenesis, along with its fundamental theoretical value, would also contribute to the development of clinical neurology.

I have no critical remarks or recommendations regarding the conducted research and the presented materials.

7. Abstract

The abstract has been prepared according to the requirements, with high quality illustrative material and sufficient volume to fully present the main results of the dissertation.

CONCLUSION

The dissertation contains results that represent *an original contribution to science* and meet all the requirements of the Law for the Development of the Academic Staff in the Republic of Bulgaria, the Regulation for its implementation, and the Regulation of MU - Varna. The presented materials and results from the scientific research fully comply with the specific requirements adopted in connection with the Regulation of MU - Varna for the application of the Law for the Development of the Academic Staff in the Republic of Bulgaria.

The dissertation demonstrates that the doctoral student Dr. Iskren Boyanov Velikov possesses in-depth theoretical knowledge and professional skills in the scientific specialty of anatomy, histology, and cytology, as well as qualities for independently conducting scientific research.

Due to the above, I give my positive assessment of the conducted research and propose to the esteemed scientific jury, appointed by Order No. P-109-222/28.04.2025 of the Rector of the Medical University – Varna, to award the educational and scientific degree of 'Doctor' to Dr. Iskren Boyanov Velikov in the doctoral program for the scientific specialty 'Anatomy, Histology, and Cytology' (03.01.02).

3.06. 2025 г.

Prepared the review:

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(Prof. Yvetta Koeva, MD, PhD)