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ACADEMIC OPINION

by **Assoc. Prof. Dr Stoyan Pavlov Pavlov, MD, PhD,** Department of Anatomy and Cell Biology, Faculty of Medicine, Medical University "Prof. Dr Paraskev Stoyanov" – Varna

Regarding: doctoral dissertation for the acquisition of the educational and scientific degree "Doctor" in the scientific specialty "Anatomy, Histology, and Cytology", professional field "7.1 Medicine", area of higher education "7. Health Care and Sports" by Dr. Iskren Boyanov Velikov, a regular PhD student at the Department of Anatomy and Cell Biology, Faculty of Medicine, Medical University "Prof. Dr Paraskev Stoyanov" – Varna, under the doctoral program "Anatomy, Histology, and Cytology," on the topic: "Role of Transcription Factor Zbtb20 in the Development of the Cerebellum" with scientific supervisor Assoc. Prof. Dr. Irina Ivanova Stoyanova-van der Laan, MD, PhD

Public defense procedure:

In my capacity as a member of the Scientific Jury according to Order No. № P-109-222/ 28.04.2025 of the Rector of Medical University "Prof. Dr. Paraskev Stoyanov" – Varna, based on a proposal from the Faculty Council of the Faculty of Medicine, and pursuant to Protocol from the meeting of the Scientific Jury No. 1/09.05.2025, I was appointed to prepare an academic evaluation statement in a procedure for the acquisition of the educational and scientific degree (ESD) "Doctor" by doctoral candidate Dr Iskren Boyanov Velikov.

Кратка професионална биография на докторанта:

Dr. Iskren Boyanov Velikov graduated with honors from high school in a specialized biology class with an emphasis on English language studies. In 2007, he obtained a Master's degree with the professional qualification of "Master Dental physician," graduating with distinction from the Medical University "I. P. Pavlov" in Plovdiv. From 2007 to 2008, he worked as a "Dental Physician" at the Rural Health Service in Razgrad region. From 2008 to 2009, after successfully passing a competition, he held the position of "Assistant Professor" at the Department of "Anatomy, Histology, and Embryology" at MU - Plovdiv. Since 2009, he has been appointed as an Assistant Professor at the Department of Anatomy and Cell Biology at the Medical University "Prof. Dr. Paraskev Stoyanov" - Varna, where he currently works. In 2013, he successfully acquired a specialty in "Anatomy, Histology, and Cytology". Since February 2019, he has been a regular doctoral student at the Department of "Anatomy and Cell Biology" at the Medical University – Varna. Over the years, Dr. Velikov has improved his skills through participation in seminars, scientific symposia, courses, and self-study. He is proficient in English at level B2 (certified by an ECL certificate).
Dr. Velikov's teaching activities are related to conducting practical exercises in all disciplines taught by the department for students majoring in "Medicine," "Dental Medicine," and college specialties, both in Bulgarian and English language instruction. His main scientific interests are in the field of neurobiology, specifically neurogenesis. He is a member of the Bulgarian Anatomical Society.

Relevance and Significance of the Dissertation Work:

The cerebellar cortex is one of the most complex parts of the brain, containing over 70% of all neurons in the central nervous system. This complex system participates in controlling higher executive functions such as motor control, procedural memory, and others. In recent years, evidence has emerged of cerebellar involvement in processes such as emotions and decision-making. Advances in computational and microscopic techniques, molecular biology, and the systemic approach in neurobiology over the past decades have begun to reveal the complexity and principles of functioning of this highly organized structure. However, these discoveries raise a new fundamental question: How does such a multilayered system form and organize itself? Understanding the molecular mechanisms that control the sequential formation, migration, and integration of different populations of neurons during development is crucial for clarifying the principles of organization in the cerebellar cortex. This knowledge will also help identify specific targets and methods for intervention that can support the prevention and treatment of various congenital and acquired diseases and conditions. In this context, the presented dissertation addresses an important and current topic of fundamental significance.

The presented dissertation examines the importance of the transcription factor Zbtb20 for the formation of the cerebellar cortex. Specifically, the scientific work investigates the effects of Zbtb20 on the development of the main classes of cells involved in the formation of the cerebellar cortex, as well as on the overall morphogenesis of the cerebellum. Methodologically, the dissertation applies a modern systemic approach, including spatial transcriptomics and proteomics methods and imaging morphological analysis, to clarify the role of the studied transcription factor in regulatory networks and its significance for the formation of the cerebellum. Using advanced multiplex microscopy imaging methods, Dr. Velikov localizes and tracks changes in certain phenotypic cell populations and their migration. By tracking the dynamics of cerebellar morphogenesis through changes in foiliation and the area of the cerebellar cortex, he demonstrates the importance of Zbtb20 for this complex process that is still not fully explained by science.

Zbtb20 is a transcription factor whose dysregulation is associated with serious socially significant neuropsychiatric disorders such as major depressive disorders and seasonal affective disorders. Its role in a number of syndromes related to developmental delays has also been proven. In this sense, the study has important clinical significance, as it can contribute to the discovery of specific mechanisms and targets for intervention in the development of therapeutic approaches for the prevention and treatment of the mentioned conditions.

Structure and analzsis of the dissertatio:

1. Contents:

The dissertation is organized according to standard requirements (literature review, materials and methods, results, discussion, conclusions, and summary). On a volume of 142 pages, 97 figures are presented, demonstrating the results of the dissertation work. Most of the figures are presented in the form of panels, and the actual number of illustrative material used amounts to more than 130 images and over 40 graphs. The sources used are adequately cited in the text. It is noteworthy that in the title of photos resulting from collective work, Dr. Velikov acknowledges the participation of other team members and confirms their permission to use the photograph, as appropriate. A total of 254 literary sources were used, all of which are in Latin script. Of these, 33% (84) are from the last 10 years, reflecting the relevance of the conducted research.

2. Literature review, Goal and Tasks:

The introduction is concise and guiding. The literature review occupies an adequate portion (29 pages) of the dissertation and is presented in a logical sequence, with a comprehensive character regarding the information available in the scientific literature. This reflects the ability of the doctoral candidate to discover, analyze, and summarize the available data on the examined problem. The literature review is divided into five parts, which sequentially and exhaustively present the latest information on the anatomy and histological structure of the cerebellum, its embryonic development, neuronal stem cells, types of cerebellar neurons, and molecular control over their development and migration. In separate sections, the known scientific information about various transcription factors and specifically Zbtb20, and their role in the pathology of the CNS and cerebellum, is summarized. The review concludes with an overview of the different glial and neuronal markers and their significance in biological, pathophysiological, and methodological aspects.

The central hypothesis and objectives are clearly formulated in a separate section. The specific tasks are appropriately formulated to achieve the goals of the doctoral candidate.

3. Material and Methods:

The materials and methods are adequately selected in a scope sufficient for realizing the study. The methodology and used materials and resources are described in sufficient detail, allowing independent scientists to replicate the experiments. The number of animals used (6 groups of 6 animals each) is sufficient for reliably establishing the observed effects of the studied transcription factor. The study uses a wide range of markers to assess the phenotype of interneuronal progenitors and the significance of Zbtb20 for their development and migration. The applied methods of imaging and spatial transcriptomics and proteomics are used adequately. The obtained images are processed and analyzed using appropriate methods that allow obtaining reliable information. The statistical methods used for analyzing and evaluating the results are adequately selected for the type of analyzed data.

4. Results and Discussion:

The original research in the "Results" section is presented systematically and illustrated convincingly over 130 photomicrographs and more than 40 diagrams. These can be summarized in the following directions: description of the expression zones and phenotype of Zbtb20 positive neuronal stem cells at different stages of cerebellar development, effects of suppressed Zbtb20 expression on the development, migration, and dynamics of cells with different phenotypes, and on the overall morphogenesis of the cerebellum (foliation). In the "Discussion" section, the results are discussed competently in light of the literature review on the problem and demonstrate excellent knowledge of the subject matter by the doctoral candidate.

5. Conclusions and original contributions:

The clearly formulated conclusions contribute to a detailed elucidation of the significance and role of the transcription factor Zbtb20 in cerebellar development. I fully accept the formulated by the author original contributions.

Recommendations: The attached article on the topic of the dissertation, authored solely by Dr. Velikov, fully meets the normative requirements for acquiring the academic degree of Doctor. However, this article is essentially a review and presents the author's research on the available literature. I recommend that Dr. Velikov prepare and publish his own experimental results in a reputable scientific journal as soon as possible.

Compliance with the criteria of the normative framework for acquiring the educational and scientific degree "Doctor":

The evidence presented by Dr. Iskren Velikov fully meets the requirements of the Law on the Development of Academic Staff in the Republic of Bulgaria and the criteria of its Implementing Rules, as well as the Rules for the Development of Academic Staff at the Medical University - Varna. In the attached academic report on similarity from the Library of MU - Varna, there is noted similarity (2% for the dissertation and 26% for the attached published article); however, this is likely due to the fact that these reports continue to be prepared on the entire text without excluding bibliographies, which is incorrect. Verification with the Strike plagiarism platform actually establishes 0% similarity with other sources in the text of the dissertation and the presented scientific publication on the dissertation topic, confirming that the dissertation is an independent work created by the doctoral candidate.

Conclusion:

The dissertation of Dr. Iskren Boyanov Velikov addresses a current topic in fundamental neurobiology and neuroanatomy. In my opinion, this is a solid scientific work that meets the requirements of the Law on the Development of Academic Staff in the Republic of Bulgaria and the Rules for the Development of Academic Staff at the Medical University - Varna for acquiring the eeducational and scientific degree of Doctor. Based on the above, I will confidently vote positively for awarding the educational and scientific degree of Doctor to the doctoral candidate Dr. Iskren Boyanov Velikov and allow myself to recommend the same to the other members of the esteemed Scientific Jury in the procedure.

Заличено на основание чл. 5, §1, б. „В“ от Регламент (ЕС) 2016/679

Date: 27.05.2025
MU–Varna

Prepared by

/Assoc. Prof. Stoyan Pavlov, MD, PhD/