

REVIEW

by Prof. Dr. Anelia Bivolarska, PhD

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Regarding: Dissertation for the award of the scientific degree “Doctor of Sciences”,

Field of Higher Education 7. Healthcare and Sports,

Professional Field 7.1. Medicine,

Doctoral Program: “Medical Biology”

Author: Prof. Maria Hristova Kazakova, PhD

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Title: “Molecular-biological and immunological studies on chitinase-like proteins CHI3L1 and CHI3L2 in processes of inflammation, tumorigenesis and neurodegeneration”

By Order No. R-1306/25.02.2026 of the Rector of the Medical University – Plovdiv and based on the decision of the first meeting of the Scientific Jury held on 05.03.2026, I have been appointed to prepare a review of the dissertation entitled: “Molecular-biological and immunological studies on chitinase-like proteins CHI3L1 and CHI3L2 in processes of inflammation, tumorigenesis and neurodegeneration”, submitted by Prof. Maria Kazakova from the same department.

BIOGRAPHICAL DATA AND PROFESSIONAL QUALIFICATION

Maria Kazakova graduated in 2005 from Plovdiv University “Paisii Hilendarski” with a Bachelor’s degree in Molecular Biology, and in 2006 obtained a Master’s degree in Cell Biology. From 2005 to 2007 she worked as a biologist at the Department of Developmental Biology at the same university. After successfully passing a competitive examination in 2007, she was appointed as an Assistant Professor at the Department of Medical Biology, Medical University – Plovdiv. Alongside her academic duties, she developed a PhD thesis in Immunology, which she successfully defended in 2013. In the same year, she obtained a specialization in Medical Biology, and the following year was appointed Chief Assistant Professor. In 2015, M. Kazakova also graduated as a Master of Applied Research Management at Plovdiv University “Paisii Hilendarski”. Since 2016 she has been an Associate Professor in Medical Biology, and since 2024 Head of the Department of Medical Biology.

ANALYSIS OF THE DISSERTATION

1. RELEVANCE OF THE SCIENTIFIC WORK

The presented dissertation addresses an extremely relevant and significant scientific topic related to the role of mitochondrial dysfunction, oxidative stress, and inflammatory processes in the pathogenesis of a wide range of socially significant diseases, including autoimmune, neurodegenerative, and tumor conditions. Contemporary scientific evidence convincingly demonstrates that these processes are key pathogenic mechanisms determining the development, progression, and complications of many chronic diseases.

Particular importance is given to the study of individual variations in cellular and molecular mechanisms, including changes in mitochondrial status and oxidative stress, which may contribute to more precise diagnostics and effective therapy monitoring. In this context, the development and validation of new biomarkers for tissue remodeling and oxidative stress represent a priority scientific task. However, such studies require significant resources, including well-characterized patient cohorts and advanced analytical methodologies.

Special attention is given to chitinase-like proteins (CLPs), which represent a novel and promising class of biomarkers. The most extensively studied member, CHI3L1 (YKL-40), is an established systemic marker of inflammation and fibrosis, elevated in various pathological conditions including cardiovascular diseases, diabetes, and asthma. Increasing scientific interest is also directed toward CHI3L2 (YKL-39), associated with chondrocyte and macrophage activity and providing more specific information on local pathological processes. Chitinase-like proteins are actively involved in the regulation of immune response, inflammation, tissue remodeling, and fibrosis, as well as tumorigenesis. Despite structural similarity, individual members of this family demonstrate different expression patterns and functional specificity, further emphasizing their diagnostic and prognostic value.

In summary, the investigation of CLPs as biomarkers of inflammation, tissue damage, and oncological prognosis is highly contemporary and of strong scientific and applied relevance. The dissertation is aligned with current trends in personalized medicine and has the potential to contribute to improved diagnostic and therapeutic approaches in chronic diseases.

2. DESCRIPTION OF THE DISSERTATION

The dissertation comprises 184 pages, illustrated with 41 figures and 31 tables. It includes 4 appendices. The bibliography contains 231 references, of which two are in Cyrillic. The high relevance of the literature is noteworthy – 52 references (26.5%) were published within the last 5 years (2021–2026), and 118 (60.2%) within the last 10 years (2016–2026).

The dissertation is structured in a clear and logical manner and includes the following main sections: introduction, literature review, aim and objectives, materials and methods, results, discussion, conclusion, summary of contributions, scientific publications related to the dissertation, and references.

The introduction is concise and introduces the reader to the core scientific problem. The literature review covers approximately 15% of the total volume and systematically presents the current state of the field. The “Materials and Methods” section accounts for approximately 13% and is comprehensively developed, providing a solid methodological foundation.

The “Results” section represents the largest part (approximately 39%), which is appropriate given the experimental nature of the work. The discussion accounts for approximately 10% and demonstrates strong analytical and interpretative capabilities. The remaining sections include conclusions, contributions, and publications.

Overall, the dissertation is well-structured, with balanced sections that facilitate comprehension of the scientific content.

3. LITERATURE REVIEW

The literature review is systematically and logically structured, covering the main aspects related to chitinases and chitinase-like proteins. A clear distinction is made between true chitinases (AMCase, CHIT1) and chitinase-like proteins, providing a strong theoretical foundation. Detailed attention is given to CHI3L1 (YKL-40) and CHI3L2 (YKL-39), including their genetic characteristics, expression patterns, and biological functions. The review integrates data from both physiological and pathological conditions, including autoimmune diseases, neurodegenerative disorders, infections of the central nervous system, and malignancies such as colorectal cancer and glioblastoma.

A strong feature of the review is the critical analysis of existing data. Differences between CHI3L1 and CHI3L2 in expression and function are clearly highlighted. While CHI3L1 is presented as a systemic marker of inflammation, fibrosis, and tumorigenesis, CHI3L2 remains less characterized, with limited and sometimes contradictory data regarding its clinical relevance. Importantly, the author identifies key scientific gaps, including the lack of consensus on CHI3L2 clinical application, absence of reference ranges, and insufficient data on their combined expression patterns. A significant emphasis is placed on the lack of data regarding the relationship between chitinase-like proteins and mitochondrial function, which justifies the present study.

Overall, the literature review is well-organized, critically oriented, and provides a solid scientific basis for the dissertation.

4. MATERIALS AND METHODS

This section is thoroughly developed and methodologically sound, fully aligned with the aims and objectives of the dissertation.

The study includes a substantial cohort of 270 participants distributed across autoimmune, neurodegenerative, and oncological disease groups. Inclusion of multiple disease entities (rheumatoid arthritis, systemic sclerosis, osteoarthritis, CNS infections, ischemic stroke, autism spectrum disorders, colorectal cancer, glioblastoma) and appropriate controls ensures a robust comparative analysis. The methodological approach is comprehensive and multidisciplinary, including clinical assessment, imaging techniques, laboratory assays, and molecular biology methods. ELISA and qRT-PCR were used for protein and gene expression analysis. Mitochondrial function was assessed using Seahorse technology, providing real-time metabolic profiling.

Statistical analyses are appropriately selected and correctly applied, ensuring validity and reproducibility of the results.

5. RESULTS AND DISCUSSION

The results section is comprehensive and corresponds fully to the aims of the study. It demonstrates elevated expression of CHI3L1 and CHI3L2 in rheumatoid arthritis and responsiveness to therapy, confirming their value as dynamic biomarkers of inflammation. In systemic sclerosis, CHI3L1 correlates with clinical indices and inflammatory markers, supporting its role in patient stratification. In CNS infections, CHI3L1 shows potential for distinguishing viral and bacterial etiologies. In oncological conditions, differential expression

patterns of CHI3L1 and CHI3L2 correlate with tumor aggressiveness. In autism spectrum disorders, a relationship between CHI3L1 and mitochondrial dysfunction is demonstrated, highlighting a novel mechanistic link.

The discussion is highly analytical and integrates molecular, cellular, and clinical data. The author demonstrates strong critical thinking and successfully interprets findings within the context of current literature.

CONTRIBUTIONS AND SIGNIFICANCE OF THE WORK FOR SCIENCE AND PRACTICE

The original contributions of the dissertation are significant and substantially enrich scientific knowledge in molecular medicine, with emphasis on CHI3L1 and CHI3L2 in inflammation, tissue remodeling, neurodegeneration, and tumorigenesis.

First, the study provides novel data on gene, protein, and tissue expression of CHI3L1 and CHI3L2 across a wide spectrum of diseases associated with chronic inflammation, neurodegeneration, and tumor progression.

Second, the identification of mitochondrial dysfunction as a key factor in neuroinflammatory and chronic inflammatory processes has important scientific and clinical implications, enabling earlier detection of metabolic disturbances and improved therapeutic monitoring.

Third, CHI3L1 is shown to promote tumor proliferation, invasion, and metastasis through modulation of inflammation and tumor microenvironment, supporting its potential as a prognostic biomarker.

The author's abstract corresponds fully to the dissertation content.

PUBLICATION ACTIVITY RELATED TO THE DISSERTATION

The scientific output related to the dissertation of Prof. Maria Kazakova includes a total of 17 scientific publications. Of these, 13 publications are indexed and referenced in internationally recognized databases (Scopus and Web of Science) and published in journals with impact factor, exceeding the minimum requirement of 10 publications. Two publications are accepted or in "submitted" status in indexed international journals.

Prof. Kazakova is first author in 6 scientific publications, demonstrating her leading role in a substantial part of the research work. According to indicator G (publications), the minimum requirement is 100 points, while 133 points have been achieved. According to indicator D (citations), 166 citations have been recorded, corresponding to 2490 points (15 points per citation), significantly exceeding the minimum requirement of 150 points. The total score is 2773 points, compared to the required minimum of 450 points, indicating a multiple exceedance of national requirements.

In addition, there is significant scientific activity demonstrated by participation in 30 scientific forums (15 national and 15 international) and involvement in 12 research projects.

CONCLUSION

The presented dissertation by Prof. Maria Kazakova entitled "Molecular-biological and immunological studies on chitinase-like proteins CHI3L1 and CHI3L2 in processes of inflammation, tumorigenesis and neurodegeneration" is relevant, original, and well-


structured, with clearly defined aims and objectives that have been successfully fulfilled. The obtained results are reliable and reproducible and are based on modern molecular, immunological, and functional methodologies. The dissertation provides new data expanding knowledge on the biological roles of CHI3L1 and CHI3L2 as key mediators linking inflammation, tissue remodeling, tumorigenesis, and neurodegeneration. The study has an interdisciplinary character and demonstrates a high level of scientific interpretation. The results provide original theoretical contributions with potential application in diagnostics, prognosis, and monitoring of diseases associated with chronic inflammation and mitochondrial dysfunction.

The publication activity, including papers in high-impact international journals indexed in global databases and participation in scientific forums, underlines the significance of the research and its recognition by the scientific community.

The merits of the dissertation and compliance with national requirements under the Law for the Development of Academic Staff in the Republic of Bulgaria and the relevant institutional regulations provide a solid basis for my positive evaluation. I recommend to the Scientific Jury that Prof. Maria Kazakova be awarded the academic degree “Doctor of Sciences” in the Field of Higher Education 7. Healthcare and Sports, Professional Field 7.1. Medicine, Doctoral Program “Medical Biology”.

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Prepared by:



Заличено на основание
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