

## R E V I E W

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External member for Medical University – Plovdiv, of the Scientific Jury, according to Order  
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REGARDING: Dissertation thesis in the scientific specialty “Medical Biology” by **Prof. Maria Hristova Kazakova-Velinova**, DSc, from the Department of Medical Biology, Faculty of Medicine, Medical University – Plovdiv, for the acquisition of the scientific degree “Doctor of Sciences”, Field of higher education 7. Healthcare and Sports, Professional field: 7.1. Medicine. Doctoral program: “Medical Biology”

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

In my capacity as a member of the scientific jury, within the procedure I was provided in due time with the documents of Prof. Maria Hristova Kazakova-Velinova, DSc

The procedure complies with the Academic Staff Development Act in the Republic of Bulgaria, its Regulations, and the Rules for Academic Development at Medical University – Plovdiv (2021)

I declare that I have no conflict of interest with the author of the dissertation thesis.

### ***Biographical data and professional qualification***

Maria Kazakova is a Professor and Head of the Department of Medical Biology at the Medical University – Plovdiv. She obtained a Bachelor’s degree in Molecular Biology (2005) and a Master’s degree in Cell Biology (2006) at Paisii Hilendarski University of Plovdiv. In 2013 she defended her PhD in Immunology and acquired a specialty in Medical Biology, and in 2015 she also completed a Master’s degree in Applied Research Management.

Her professional career includes positions from biologist and assistant to associate professor and professor, with active participation in academic and research councils of the university. She delivers lectures and practical training in parasitology, molecular biology and immunology to students of medicine, dental medicine and pharmacy in Bulgarian and English, and is author and co-author of teaching materials and manuals.

She has extensive experience in leading scientific projects – over 29 national and international, and specializations in Bulgaria, Italy and Russia, including leadership training at Harvard University (2023–2024). She is a member of the Union of Scientists in Bulgaria and the European Immunology Society, actively participates in academic juries, state examinations and the training of PhD students and residents. She is fluent in English and Italian, with proven communication and management skills.

### ***Characteristics and evaluation of the dissertation thesis and contributions***

#### **1. Relevance and knowledge of the researched topic**

The dissertation thesis is structured in the following sections: introduction, literature review, aims and objectives, materials and methods, results, discussion, conclusions and

contributions. The bibliography contains 210 titles, about 30% of which are from the last 5 years. At the end of the dissertation thesis, 4 appendices are systematized.

The presented dissertation work is devoted to a relevant and multidisciplinary scientific problem, focused on the molecular-biological and immunological aspects of chitinase-like proteins CHI3L1 and CHI3L2 in the context of inflammation, tumorigenesis and neurodegenerative processes. The topic is contemporary and significant, as it integrates key directions in biomedical research – immunology, molecular biology, neuroscience and oncology, with direct relevance to the diagnosis, prognosis and therapeutic monitoring of socially significant diseases.

The literature review demonstrates very good knowledge of the state of the scientific problem. Contemporary literature sources are thoroughly analyzed, covering both fundamental aspects (structure, gene organization, biological functions) and clinical applications of the studied molecules. The role of chitinases and chitinase-like proteins as part of the GH18 family, as well as their involvement in key pathophysiological processes such as inflammation, tissue remodeling, angiogenesis and immune regulation, are presented in a consistent and logical manner. The role of CHI3L1 and CHI3L2 is described in detail in several diseases with different etiology such as rheumatoid arthritis, systemic sclerosis, osteoarthritis. Data on the levels of the two chitinase-like proteins in ischemic stroke, autism spectrum disorder and infections of the central nervous system are systematically presented. A separate chapter is dedicated to the significance of these proteins in tumorigenesis, and more specifically in colorectal carcinoma and glioblastomas.

Unresolved scientific questions are clearly formulated, including the lack of consensus regarding the role of CHI3L2, insufficient data on the co-expression of the two proteins, as well as the absence of established reference values and their relationship with mitochondrial function. These gaps in knowledge convincingly justify the aims and objectives of the dissertation thesis.

In conclusion, the topic of the dissertation thesis can be assessed as highly relevant, scientifically significant and with potential contribution both in theoretical and applied aspects.

## 2. Methodology of the study

The section “Materials and Methods” is written on 24 pages. The presented materials and methods demonstrate a well-planned, multidisciplinary and modern research approach, corresponding to the set objective – a comprehensive study of CHI3L1 and CHI3L2 in various pathological processes with a strongly expressed translational design. The cohort of 270 patients is relatively large and diverse, covering autoimmune, neurodegenerative and oncological diseases, which is a strength, as it allows broad interpretation and comparability between different pathogenetic models. The presence of control groups for each nosology further increases the validity of the results, although in some subgroups (e.g. CNS infections, RA, SS) the number of controls is relatively limited.

The inclusion and exclusion criteria are clearly defined and comply with contemporary diagnostic standards (ACR/EULAR, DSM-5, NIHSS). The clinical and imaging methods used (ultrasound, MRI, CT, standardized scales) are adequate and fully

applicable to the respective diseases.

The laboratory methods are modern and widely established. A particularly strong aspect is the study of mitochondrial function using Seahorse technology. This type of analysis adds value to the work, significantly increasing its originality.

### 3. **Results and their interpretation**

The “Results” section is the most extensive, written on 72 pages. The text is clearly structured, progressing from demographic and clinical characteristics of the patients to an in-depth analysis of biochemical, molecular and mitochondrial parameters, which allows tracing the relationships between different levels of organization – from gene expression to functional response.

The results are presented systematically by combining descriptive statistics, comparative analyses and correlation studies, with methods appropriate to the data distribution. The non-normal distribution of a significant part of the variables is correctly accounted for, and non-parametric tests are accordingly applied, demonstrating good statistical competence. The inclusion of cluster analysis in patients with CNS infections significantly enriches the interpretation and allows identification of different clinical-biological subtypes, which is a substantial contribution. Similarly, in ASD, the use of ROC analysis and logistic regression for patient stratification adds predictive value to the results and demonstrates a pursuit of clinical applicability.

The visualization of the results is at a very high level. The text includes 41 figures and over 31 tables, presenting both raw data and summarized trends. Various visualizations are used – box plots, bar charts, correlation dependencies, cluster profiles, as well as schematic representations of biological processes. This diversity facilitates the understanding of the information and allows clear tracking of the main relationships. The tables are informative and contain key statistical indicators such as median, interquartile range, mean value and standard deviation, allowing a full assessment of data distribution. Particularly impressive is the integration of different types of data – clinical, laboratory, gene and functional – into a unified analytical framework.

For the first time, data on CHI3L2 in the context of ischemic stroke are presented and an attempt is made for functional interpretation of these results, which has potential scientific novelty. A strong aspect is also the comparative nature of the analysis across different diseases, allowing the derivation of more general pathophysiological patterns.

The conclusions drawn are consistent with the obtained results and do not exceed their evidential value. The overall impression is of a well-conducted and in-depth study with high scientific value, contributing to the understanding of the role of CLPs in various pathological processes.

The presented discussion is largely consistent, logically structured and well connected to the obtained results, successfully integrating the data into a broader context of current knowledge about the role of CHI3L1 and CHI3L2 in autoimmune, neurodegenerative and oncological diseases. The interpretation of the results is well-argued and supported by literature sources, creating a sense of scientific validity and

reliability. The link between experimental findings and known pathophysiological mechanisms is clearly traced, with a particularly strong impression made by the interdisciplinary approach and the attempt to integrate immunological, molecular and mitochondrial aspects. Particularly valuable is the effort to interpret unexpected or contradictory results, such as the relationship between CHI3L1 and mitochondrial reserve capacity, demonstrating critical thinking and scientific maturity.

#### **4. Contributions and scientific significance of the dissertation thesis**

Among the main scientific contributions of Prof. Kazakova's dissertation is the comprehensive study of CHI3L1 and CHI3L2 at several levels – protein (plasma and cerebrospinal fluid), gene expression and local tissue expression, allowing an overall assessment of their biological role. It is demonstrated that CHI3L1 functions as a sensitive indicator of inflammation, tissue damage and tumor activity, while CHI3L2 exhibits more specific expression characteristics associated with tumor aggressiveness. In this regard, it is established that the two proteins show different expression patterns in colorectal carcinoma and glioblastoma, associated with the degree of malignancy and disease progression.

In autoimmune diseases, significant data are obtained regarding the role of CHI3L1 as a marker of inflammatory activity. In patients with rheumatoid arthritis, significantly increased gene and protein expression of CHI3L1 and CHI3L2 is demonstrated before initiation of therapy, followed by a decrease depending on the applied treatment, reflecting reduction of the inflammatory process. Similarly, in systemic sclerosis, increased plasma levels of CHI3L1 and their association with clinical parameters and pro-inflammatory cytokines confirm its role as a marker of chronic autoimmune inflammation and as a tool for patient stratification.

A substantial contribution is also the analysis of tumor tissue in colorectal carcinoma, where a statistically significant increased expression of CHI3L1 and CHI3L2 is established, especially at the invasive front and in tumor budding. The combined assessment of tissue expression of CHI3L1 and the degree of tumor budding emerges as a reliable indicator of the metastatic potential of the tumor and of processes of tumor invasion and cellular communication. Additionally, in glioblastoma, a dynamic change in serum levels of CHI3L1 in the early postoperative period is demonstrated, highlighting its association with the acute inflammatory response and tissue reactivity. In the field of neuroinfections, it is established that CHI3L1 expression has diagnostic value, as it can support early differentiation between viral and bacterial infections of the central nervous system, which has important clinical significance for timely therapeutic management.

Another key contribution is related to the study of mitochondrial function using Seahorse technology, where for the first time in a Bulgarian cohort a detailed bioenergetic profile is presented across different diseases. It is established that mitochondrial dysfunction has a disease-specific character – pronounced in rheumatoid arthritis, minimal in ischemic stroke and with a specific profile in autism spectrum disorders. In the latter, increased reserve respiratory capacity and maximal respiration are observed, suggesting impaired metabolic adaptation and increased

sensitivity to oxidative stress. In this context, it is shown that CHI3L1 correlates with mitochondrial parameters and clinical scales in patients with ASD, allowing its consideration as a potential marker of mitochondrial dysfunction.

Additionally, the study reveals new aspects of the relationship between CHI3L1, CHI3L2 and mitochondrial function, especially in rheumatoid arthritis, where CHI3L1 correlates with reserve respiratory capacity and indicators of energy efficiency. This expands the understanding of the role of these proteins beyond classical inflammatory mechanisms, positioning them also as potential mediators of cellular metabolism.

Last but not least, the developed stratification model in patients with autism spectrum disorders, based on mitochondrial parameters, represents an innovative approach with potential clinical application, demonstrating the possibility to distinguish patient subgroups, including in the presence or absence of regression.

In summary, the dissertation thesis provides new and original data on the multifactorial role of CHI3L1 and CHI3L2 in the pathogenesis of inflammatory, neurological and oncological diseases, linking them both to the immune response and to cellular metabolism and tumor progression. The obtained results substantiate their potential as biomarkers for diagnosis, stratification and monitoring of therapeutic response, as well as possible therapeutic targets, emphasizing the importance of an integrated multidisciplinary approach in the study of complex diseases.

#### 5. **Abstract**

I find that the abstract adequately and fully reflects the content and the main merits of the dissertation thesis.

#### 6. **Critical remarks**

A main limitation is the small sample size in individual groups, which reduces the statistical power and generalizability of the results. The lack of significant correlations in some analyses is likely due to this. To some extent, I find it problematic the presence of potential bias in the controls – use of postmortem cerebrospinal fluid (highly problematic) and different types of controls across groups. For some indicators (e.g. CHI3L2 in cerebrospinal fluid, ASD protein levels), the data are limited or below the detection threshold, which complicates interpretation.

#### 7. **Scientometric analysis of publications and scientific activity**

The results of the conducted comprehensive study are presented in 17 scientific publications, of which 14 are published in journals with impact factor (IF). Four of these publications are in prestigious Q1 journals with high impact factor, such as *Int. J. Mol. Sci.* (IF = 4.9) and *Frontiers in Immunology* (IF = 5.9). The total number of citations is 166. The data from the dissertation thesis have been presented at a total of 30 scientific forums, of which 15 are international. The implementation of the dissertation thesis has been supported financially by several intramural university projects, two national and one European project.

### **CONCLUSION**

The dissertation thesis “Molecular-biological and immunological studies on chitinase-like

proteins CHI3L1 and CHI3L2 in processes of inflammation, tumorigenesis and neurodegeneration” presents an in-depth study and contributes new knowledge on the biological function of CHI3L1 and CHI3L2 and their relationship with clinical parameters, expanding the possibilities for an integrated approach in medicine. Prof. Maria Kazakova not only meets the requirements but significantly exceeds them. She has 2,773 points compared to the minimum required 450 points, thereby fulfilling all requirements for the degree “Doctor of Sciences” according to the Rules for Academic Development at Medical University – Plovdiv. Based on the above, **I give a positive evaluation and vote “in favor of”** awarding the scientific degree “Doctor of Sciences” in field 7. Medicine, doctoral program “Medical Biology”.

11.04.2026

Reviewer: .....  
/Prof. Snezhina M. Kandilarova/

Заличено на основание  
Чл.5 §1, б.“В” Регламент (ЕС)2016/679