БЪЛГАРСКА АКАДЕМИЯ НА НАУКИТЕ
И-Т ПО ЕКСПЕРИМЕНТАЛНА МОРФОЛОГИЯ, ПАТОЛОГИЯ И АНТРОПОЛОГИЯ С МУЗЕЙ
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СОФИЯ

OPINION

by Prof. Mashenka Borissova Dimitrova

Member of the Scientific Jury, appointed by Order RD-15-27 of the Director of IEMPAM-BAS

Subject: Dissertation for obtaining the scientific degree "Doctor of Science" in the specialty "Biochemistry" (01.06.10) from Professional field 4.3 "Biological Sciences" on "Biochemical basis of Alzheimer's disease" by Prof. Ludmil Penuv Kirazov.

Prof. Kirazov has presented a full set of excellently made and neatly arranged documents, in full compliance with the requirements of Law for Development of the Academic Staff in the Republic of Bulgaria (LDASRB) and its Regulations.

Ludmil Penuv Kirazov was elected as Professor of Biochemistry (01.06.10) in 2016 at IEMPAM-BAS after successfully applying for a competition. His biographical data show an impressive scientific career with numerous specializations in Germany, publications in prominent international and national journals, presentation of results at scientific forums in Bulgaria and abroad and a large number of noticed citations (over 450, h = 9, total IF 43.32). Recognition for his qualities not only as a scientist, but also as an administrator is his election as Chairman and participant in the commissions of the National Science Fund at the Ministry of Education and Science (2017-2021) and as Chairman of the General Assembly of Scientists at IEMPAM (2019 until now). A reference to the requirements for acquiring the scientific degree "Doctor of Sciences" from the Regulations of IEMPAM for application of the LDASRB shows 652 points, which significantly exceeds the threshold minimum of 350 points.

The thesis presented for opinion is written on 280 pages and contains the standard sections, with the most space devoted to the presentation of the results and their discussion (150 pages). The results are illustrated with 9 tables and 86 figures of excellent quality.

The dissertation is devoted to a topical issue - a study of the biochemical basis of the pathogenesis of Alzheimer's disease (AD). Despite numerous publications on the problem, the etiology of this severe progressive neurodegenerative disease is still unclear, which is why there is a lack of adequate treatment and so far mainly maintenance therapies are applied.

The literature review is written concisely and purposefully. The membrane form of amyloid precursor protein (APP), its processing, amyloid beta peptide and its forms (Aβ), τ-protein, as well as the hitherto known role of these components in norm and AD are described. The potential factors for the development of AD and the used model systems for the study of the disease are indicated. Special attention is paid to the mixed pathology of AD, which is the most important reason for the lack of effective treatment. Data from 700 sources were used, which is a reflection of the topicality of the issue, but also of the excellent awareness of prof. Kirazov.

The used materials and the methods applied are described in sufficient detail to allow the experiments to be repeated. The huge set of biochemical, immune, genetic and other methods used by Prof. Kirazov is impressive. At each stage of the work there is a detailed analysis of the effectivity of the applied method, improvements have been made or other methods have been tested, which shows high precision and a strong desire to obtain the most reliable results. A good example is a comparative study of two methods for determining protein (Lowry and Bradford) to find out which one is more suitable for membrane-bound proteins. Also, tissue sections were

used for quantitative analysis of APP and its degradation products (Aβ) released under different conditions, but also a method developed by Prof. Kirazov for isolation of growth cones and synaptosomes. The dissertation abounds with examples of searching for and finding the best experimental procedures for each specific study. It should also be noted the application of many modern research methods such as culturing neurons on a network of microelectrodes, isolation, sequencing and mapping of RNA transcripts, RT-PCR and RT-qPCR, bioinformative identification of linear and circular RNA, experiments with transgenic laboratory animals. The abundance of experimental methods and their precise application have led to the achievement of significant scientific results.

The results and discussion are presented very clearly and consequently. The motivation for conducting each experiment is based on data from the literature and / or own observations. After each result, a conclusion is given, which outlines its significance and logically leads to the next study.

Data were obtained on the importance of glutamate receptors for APP secretion and on its processing by calcium-dependent proteases, as well as on the absence of feedback between the amount of soluble APP and APP secretion. The importance of vascular endothelial growth factor (VEGF) for APP processing has been studied in details and has been shown that it leads to a transient inhibition of Aβ-fibrillogenesis. IL-1β has been shown to play a significant role in the cholinergic deficiency observed in patients with AD, by suppressing intracellular transcription factors that may become potential targets for future therapies.

The study on the importance of cholinergic denervation for the pathogenesis of AD is impressive. For this purpose, transgenic mice with AD were used, in which cholinergic immunolesia of the basal ganglia with immunotoxin was induced. This treatment has led to the development of a pathology very close to that observed in people with AD. It can be said that a way has been found to improve the animal model of AD to be used in future research. Studies with this model cover a wide range of biochemical factors and lead to the conclusion that cholinergic denervation is crucial for amyloidogenesis. This result supports future research on the treatment with cholinomimetics.

Regarding the physiological role of APP, no clarity has been achieved so far. Prof. Kirazov's contribution in this aspect is based on studies on homogenates, isolated growth cones and synaptosomes from embryos, which convincingly show the role of APP for synaptogenesis and maintenance of normal synaptic function. These results are also confirmed by studies on the expression of mRNA of various isoforms of APP in ontogenesis, which has allowed to determine which one of them is the key factor for the formation of synaptic connections. The studies have been extended to peripheral tissues, showing the importance of APP for their development.

An important scientific contribution of Prof. Kirazov is the application of the model of "neural networks cultured on a network of microelectrodes" to establish the role of various Aβ-peptides in suppressing the electrical activity of neurons. The application of this model suggests that the action of Aβ-peptides is mediated by receptors, and more detailed studies draw attention to GABA-receptors. It also allowed to obtain data in favor of the hypothesis of the participation of monomeric soluble Aβ in the pathogenesis of AD, according to which the formation of plaques reflects the "desire" of the body to neutralize toxic Aβ.

For the first time, detailed studies of the synaptosomal transcript in young and adult animals have been performed to determine differences that would point to the pathogenesis of

AD, as the disease is predominantly characteristic of the elderly. Long intervening non-coding RNAs and circular RNAs characteristic of brain aging have been found, and their human orthologues have been sought. The study was conducted with great precision, which allowed to identify more RNAs with altered expression in aging than previously known. These results provide the basis for future research on the molecular basis of AD.

Data were obtained about the suppression of APP levels from lead salts, which made it possible to propose a mechanism to explain the neurotoxic effects of lead.

The above-mentioned essential moments from the scientific work of Prof. Kirazov do not cover the whole variety of experimental approaches, studied factors, conclusions and scientific contributions related to the biochemical bases of AD.

On the basis of the conducted experiments, conclusions have been made and scientific contributions have been outlined, with all of which I agree. Scientific contributions are divided into original and applied. I dare say that the applied contributions are also original.

The abstract correctly reflects the content of the dissertation.

Conclusion: The scientific thesis of Prof. Ludmil Kirazov presents a comprehensive, in-depth and precise study, which greatly enriches the knowledge about the biochemical mechanisms of the pathogenesis of Alzheimer's disease. The results obtained are valuable; they reveal prospects for future research and identify potential targets for the development of novel therapies. Both the work and the scientometric indicators of Prof. Kirazov fully comply with the requirements of LDASRB and its Regulations.

Based on the above, with deep conviction I recommend the honorable Scientific Jury to award Prof. Ludmil Penuv Kirazov the degree “Doctor of Science” in Biochemistry (01.06.10) from the Professional Field 4.3 "Biological Sciences".

05/17/2021 Signature:

(Prof. M. Dimitrova)