

## STATEMENT OF OPINION

by **Victoria Sarafian, MD, PhD, DMSc,**

Professor of Immunology at the Medical University of Plovdiv,  
Specialist in Clinical Immunology and Medical Biology,  
at the Department of Medical Biology.

Regarding the thesis for the award of the educational and scientific degree "**PhD**":

Professional field - **Medicine**

Doctoral program – **Psychiatry**

**Author:**

**Anna Aleksandrova Todeva-Radneva, MD - PhD student on independent  
preparation**

Topic: " Translational cross-validation of neuroimaging and molecular  
biomarkers in the differential diagnosis of unipolar and bipolar depression "

**Scientific advisor:** Assoc. Prof. Sevdalina Kandilarova, MD, PhD

**Consultant:** Priv.-doz. Ronald Sladky, PhD

### **General data**

The review was prepared following Order №P-1756/06.06.2024 of the Vice-Rector of the National Research Institute of Medical University - Plovdiv on the basis of the submitted dissertation on electronic media. The reading of the dissertation leaves the overall impression of a conscientiously and competently written thesis, structured according to the rules for the preparation of such. It is a study developed in volume and content, meeting the requirements for a dissertation, in the course of which the doctoral candidate has enriched her scientific knowledge not only in the field of psychiatry. She has gained undoubted experience in her profiled training in imaging, neuroimaging analysis and has become familiar with basic principles in molecular biology and diagnostics.

The thesis is structured according to the rules of dissertation writing with an optimal balance between its different parts. In its present form, the individual sections are presented as follows: introduction - 1.5 pages; literature review - 19 pages; research hypothesis - 1 page; aim and objectives - 1 page; material and methods - 30.5 pages; results - 32 pages; discussion - 22 pages, conclusions - 1 page; contributions - 1 page and 246 references cited on 2 pages.

## **Topic relevance**

The supervisor and the PhD student have chosen an interesting topic in translational neuroscience and have set themselves ambitious tasks of an interdisciplinary nature. The trend of increasing prevalence of psychiatric diseases, including affective disorders, the lack of fully validated diagnostic approaches, the overlap of symptoms with other pathologies and the lack of highly specific biomarkers determines the relevance of Dr. Anna Todeva's dissertation.

## **Knowledge of the problem**

The literature review shows a good knowledge of the etiopathogenetic mechanisms and diagnostic approaches in the differential diagnosis of unipolar and bipolar depression, which is supported by an analysis of the literature cited. It is written in a clear, systematic and specific manner.

Particular attention is paid to current epigenetic signatures that, along with neuroimaging parameters, could aid in patient stratification and differential diagnostic refinement.

It is pointed out that the available literature data are extremely heterogeneous and not sufficient to lead to a change in the nosological nomenclature and in the diagnostic and therapeutic approach in affective disorders. This calls for a comprehensive study of etiological and pathogenetic mechanisms through a multimodal and multidisciplinary approach.

The ultimate goal from a clinical perspective is cross-validation between neuroimaging and molecular genetic analyses and translation into clinical practice.

The **scientific hypothesis** of the study is broken down into several separate hypotheses based on a multimodal and transdisciplinary approach.

The **aim** is logically derived and sounds specific and focused.

The **tasks** as content correspond to the aim and to the planned own studies. They are presented in a generalized way and imply the application of new statistical methods to process already collected neuroimaging data and the implementation of a newly developed fMRI paradigm in an independent sample. The innovative potential of this dissertation lies precisely in the realization of these tasks. Probably due to a technical translation problem, the long non-coding RNAs (lncRNAs) in task 3 are written as dncRNAs, which is not consistent with the content. For convenience and correctness, the two types of molecules could be referred to by their common English names, lncRNAs and miRNAs.

## **Material and methods**

This dissertation chapter is written with the necessary precision, accuracy and detail and allows for easy reproducibility and repeatability of results. Ethical aspects, inclusion and exclusion criteria for patient selection are accurately described.

The study was large and included 103 subjects, 43 healthy controls, 35 patients with major depressive disorder and 25 with bipolar disorder. The second target group consisted of 78 individuals - 40 healthy controls, 23 patients with major depressive disorder and 15 with bipolar disorder. The study was approved by the Scientific Ethics Committee of Medical University - Plovdiv.

The methods used are informative and adequate for the set tasks. They combine clinical, neuroimaging and laboratory tests. The latter are based on isolated total RNA from white blood cells and circulating and exosomal plasma mRNA, followed by precise molecular biological analyses.

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

The results are illustrated by 11 tables and 16 complex figures, which present the research and its results in stages. First, the socio-demographic data and those from the neuroimaging analysis of the retrospective and prospective sample are presented.

The results of the molecular biology analyses did not reveal statistically significant differences in the expression level of the selected long-strand noncoding RNAs at the intergroup level.

Expression levels of selected miRNAs were examined in a sample of 14 healthy controls, 11 patients with major depressive disorder, and 16 patients with bipolar disorder. There was a statistically significant decrease in the transcriptional levels of let-7f, miR-30c, miR-212, miR-219, miR-125b, miR-126, miR-138, miR-146a and miR-182 in the patient group. In bipolar depression, the expression levels of let-7f, miR-30c, miR-212, miR-219, miR-125b, miR-126, miR-138, miR-146a, and miR-182 were decreased compared with healthy controls. Transcriptional levels of let-7f, miR-212, miR-219, miR-126, miR-138, miR-146a and miR-182 were also lower. An interesting finding was the lack of a statistically significant difference in the expression levels of selected miRNAs between unipolar and bipolar depressed patients.

The differential expression of miRNAs relevant to the processes of neurodevelopment, neurogenesis, neurodegeneration and neuroinflammation in patients with depression offers a possible explanation for their heterogeneous

clinical presentation. Some of the miRNAs studied are associated with clinical symptomatology also found in other psychiatric and neurological disorders. The lack of significant difference in miRNA expression suggests common molecular mechanisms in major depressive disorder and bipolar disorder.

The **discussion** follows the study design and shows an analytical comparison with literature data and own results. This is the most difficult part of any research paper and demonstrates not only the researcher's awareness of the problem but also his or her ability to critically, objectively and thoroughly draw conclusions or formulate original hypotheses. In this part of the dissertation, Dr. Todeva has demonstrated depth and knowledge. The discussion has been greatly expanded and enriched with new information and gives a complete and analytical appearance to the entire dissertation. The PhD student shows scientific competence in her narrow specialty, but also basic knowledge in the interdisciplinary fields relevant to the topic under development.

The **conclusions** of this innovative interdisciplinary work follow the set objectives and are based on the original data obtained.

I accept without remarks the mentioned original theoretical-methodological and scientific-applied **contributions**.

The **abstract** correctly reflects the content of the thesis.

## **Publications**

Dr. Todeva has submitted a list of 3 scientific publications. Two of them are reviews in Current topics in medicinal chemistry with IF - 3.57 and the third is an original article in Biomedicines with IF - 4.757. I would recommend that the IF of the journals and their quartiles be listed in the abstract.

The results of the study have been presented to the scientific community at 3 national to 2 international forums.

My personal impressions of the work of Dr. Todeva are related to the preparation of a joint project proposal to the FNR and MU-Plovdiv. She showed a committed and competent attitude, as well as the ability for critical analysis and good professional awareness.

I hope that A. Todeva will maintain and develop her scientific interests after the defense of her dissertation.

## **CONCLUSION**

The dissertation of Dr. Anna Todeva-Radneva entitled "Translational cross-validation of molecular and neuroimaging biomarkers in the differential diagnosis

The dissertation of Dr. Anna Todeva-Radneva entitled "Translational cross-validation of molecular and neuroimaging biomarkers in the differential diagnosis of unipolar and bipolar depression" addresses a hot scientific problem by using highly specialized and informative methods and offers original results with scientific application. The content and contributions of the dissertation meet the requirements of the Law on the Development of Academic Staff in the Republic of Bulgaria and the Regulations of the Medical University - Plovdiv for the Acquisition of the Educational and Scientific Degree "PhD".

The dissertant and her supervisor have successfully implemented a large-scale and ambitious interdisciplinary study.

Due to the above, I give my positive evaluation for the conducted research and propose the members of the scientific jury to award the educational and scientific degree "PhD" in Psychiatry to Dr. Anna Todeva - Radneva.

29.07.2024

Reviewer:

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

**Prof. Dr. Victoria Sarafian, PhD, DMSc**