OPINION

by Prof. **Albert Ivanov Krastanov**, D.Sc, Head of the Department of Biotechnology, University of Food Technologies (UFT) – Plovdiv

of dissertation for awarding the scientific degree "**Doctor of Science**" Field of higher education: 4 "Natural Sciences, Mathematics, and Informatics" Professional field: 4.3 "Biological Sciences" Scientific specialty: "Molecular Biology"

Author: Assistant Professor **Tihomir Iliev Vachev** - Plovdiv University "Paisii Hilendarski".

Dissertation thesis: "*Comparative genomic, transcriptomic and proteomic studies in neurodevelopmental disorders*"

Tihomir Iliev Vachev was born in 1981 in the town of Kardzhali. In 1999 he completed his secondary education at the, High School Otets Paisii - Kardzhali. He obtained a bachelor's degree in Biology in 2004 at the Faculty of Biology of Plovdiv University "Paisii Hilendarski", followed by a master's degree in "molecular biology and biotechnology". In 2007 he started working as a biologist junior expert at the University of Plovdiv "Paisii Hilendarski", Department of Plant Physiology and Molecular Biology. He started working as an assistant in 2011. In 2011, he successfully defended his dissertation in the doctoral program Molecular Biology - Plovdiv University "Paisii Hilendarski".

In 2013, the candidate won a competition for a assistant professor at the Department of Plant Physiology and Molecular Biology, University of Plovdiv "Paisii Hilendarski". In 2016 he defended his PhD in Genetics at the Medical University - Plovdiv. As a university lecturer, he gives lectures on an introduction to molecular biology in the bachelor's degree in molecular biology, and practical classes on gene expression regulation, molecular virology, and molecular genetics, as well as lectures on molecular medical diagnostics in the master's degree in applied molecular biology.

Asst. Prof. Tihomir Vachev has participated, representing the University of Plovdiv and in particular its Faculty of Biology, in dozens of scientific forums at home and abroad. Asst. Prof. Tihomir Vachev is a participant in the research teams of dozens of university and national projects. He is currently the author of 38 scientific publications, with an h-index of 8. He is the author of 3 manuals for students.

The dissertation thesis is discussed and aimed to dissertation defense in the meeting of the extended Departmental Council (Protocol №10 / 29.11.2019) of the "Department of Plant Physiology and Molecular Biology" at the Biology Department of University of Plovdiv" Paisii Hilendarski''.

The research included in the dissertation is funded within projects:

- "Autism and specific speech development disorder - comparative genomic studies to identify a common pathogenetic basis"№ NFSR-B01/21 at the Department of Pediatrics and Medical Genetics, MU - Plovdiv.
- "Study of the methylation status of candidate genes associated with the autism spectrum disorder in order to elucidate the epigenetic mechanisms involved in its pathogenesis" № NO-09/2014.
- "Analysis of the expression profile of non-protein-coding - microRNA genes in schizophrenia" and respectively project.
- "Study of serum levels of microRNA biomarker molecules in children with autism in order to create a platform for non-invasive molecular diagnosis of autistic patients" № NO -12/2013.

The set of materials presented in electronic format by Asst. Prof. Tihomir Vachev is in accordance with the Regulations for the development of the academic staff of the University of Plovdiv and includes the following documents:

- Request to the Rector of University of Plovdiv "Paisii Hilendarski" for disclosure of the protection procedure;

- CV in European format;

- Notarized copy of the diploma for higher education (Master's degree);

- Documents from departmental councils, related to reporting of readiness for opening the procedure and preliminary discussion of the dissertation;

- Dissertation work;

- Abstract (in English and Bulgarian);

- List of scientific publications on the topic of the dissertation;

- Copies of scientific publications;

- Declaration of originality and authenticity of the attached documents;

- Reference for covering the minimum national requirements in direction 4.3. Biological sciences. for obtaining the scientific degree "Doctor of Sciences"

2. Relevance of the topic

According to the World Health Organization (WHO), hundreds of millions of people around the world suffer from mental illness. These include debilitating diseases such as schizophrenia, Autism Spectrum Disorder (ASD), Specific language impairment (SLI), etc., which cause enormous weight, loss of productivity to affected users, relatives, and the health system. The term "autism spectrum disorders" (ASD) refers to a wide range of

developmental disorders, which are characterized by a complex and unclear etiology. Schizophrenia is another severe multifactorial psychiatric illness with a population frequency of approximately 1%, which is often chronic and is associated with affecting almost all higher cortical functions: social, behavioral, cognitive, and emotional. Many of the classic symptoms are present in other psychiatric illnesses, making it difficult to accurately diagnose. The reasons for the development of schizophrenia still remain unclear, although it is known that it is the result of a complex interaction between genetic predisposition (affecting a number of genes, each of which in itself has a weak effect) and environmental factors.

Recent advances in molecular genetics and biotechnology, the development and application in recent years of new next-generation sequencing technologies as well as the development of analytical techniques for proteomic analysis, have enabled the discovery of a large number of molecular candidate biomarkers. Due to the availability of standardized methods for characterization and profiling of mRNA molecules (RNA Seq) and miRNA molecules (Small RNA sequencing) as well as fast and universally applicable approaches to quantitative analysis (quantitative RT-PCR) suggests that the approach to identify mRNA and miRNA as potential biomarkers from their initial identification to their individual validation would be even more effective than the validation approaches of traditional protein biomarkers.

Proteomics is another potential approach that can generate new hypotheses essential to the pathogenesis and diagnosis of disease by identifying candidate protein biomarkers. Proteomic approaches complement genetic and genomic research and thus focus attention on protein products. The technologies presented by the dissertation have the potential to lay the foundation for the beginning of early diagnostic testing, detection, and assessment of disease development by generating information about the pathogenesis of disorders, all of which are essential for the successful diagnosis and development of future therapies, especially in patients, where timely therapeutic interventions are extremely critical.

From the dissertation submitted for review, my opinion on the knowledge of the problem is excellent. The Asst. Prof. Tihomir Vachev knows in detail the problem which I can judge from the presented bibliography which is extremely detailed. The review and discussion are written professionally and in detail, allowing for a step-by-step entry into the details of the topic. The dissertation cites and discusses contemporary hypotheses and opinions on the issue related to the etiology and pathogenesis of neurodevelopmental disorders, their current level of research, and guidelines for future research. Additional focus is made on the level of modern molecular genetics research in the field. With all that has been said so far, I believe that the dissertation knows in detail the problem which gives me the right to define him as a specialist in the field.

4. Research methodology

In order to meet the goals set in the research, the dissertation sets the following tasks, behind the implementation of which are the methods used in this paper.

1. Conducting a comparative expression analysis of protein-coding geniuses (RNA Sequencing) in ASD.

2. Conducting a comparative expression analysis of small RNA molecules (Small RNA sequencing) in ASD.

3. Conducting a comparative proteome analysis - Isobaric Tag for Relative and Absolute Quantification (ITRAQ) in ASD.

4. Carrying out large-scale exome sequencing (Whole Exome Sequencing) in ASD.

5. Conducting a comparative expression analysis of protein-coding geniuses (Digital Gene Expression) in schizophrenia.

6. Conducting comparative expression (qRT-PCR) analysis of protein-coding geniuses in ASD.

7. Conducting comparative expression (qRT-PCR) analysis of miRNA in ASD.

8. Conducting comparative expression (qRT-PCR) analysis of protein-coding genes in schizophrenia.

9. Conducting ROC (Receiver Operating Characteristic) analysis of differentially expressed protein-encoding genes and miRNA molecules in ASD and schizophrenia.

In the presented dissertation the author describes in detail the method of blood sampling, the method of secretion of samples, the methods for extraction of total RNA from peripheral blood, and the method for extraction of total DNA from peripheral blood. The principle of the procedure and the individual stages are consistently and completely described in detailed protocols, and the results are presented in tables. The author performs RNA sequencing (Transcriptome Analysis - Methodology) by performing qualitative assessment and filtering of sequencing data, quantitative analysis of gene expression and study of gene coverage, as well as the levels of gene expression of candidate genes.

To further validate the obtained results, the author uses the method Real-Time qRT-PCR (Quantitative Reverse Transcription Real-Time PCR) for quantitative analysis of the obtained data. The method allows for reliable detection and measurement of the generated PCR products in each cycle of the polymerase chain reaction process. The stages of their implementation, as well as the applied methodology and equipment, are precisely selected and described in detail. The obtained results are presented in illustrative figures.

For analysis of the protein profile in ASD, the Asst. Prof. Tihomir Vachev uses quantitative proteomic analysis - Isobaric Tag for Relative and Absolute Quantification

(ITRAQ), which indicates the principle of quantitative proteomic analysis (iTRAQ) analysis, emphasizing the advantages of the iTRAQ method for the purposes of his research.

The author also applies an approach for complete genomic sequencing of all protein-coding regions. According to the dissertation, the labor-intensive approach to complete sequencing of all coding regions has the potential to become both a clinically relevant test for genetic diagnosis and a means of identifying DNA variants with a potentially significant pathological effect in diseases of unknown etiology. The aim of this approach is to identify functional changes in the genome that are responsible for the development of a specific pathology. The large-scale exome sequencing used by the dissertation is fast becoming the technique of choice when it comes to identifying new genetic variations that underlie diseases such as a number of cancers and a number of psychiatric diseases such as schizophrenia, ASD, and others.

One key feature of identifying specific DNA variants (SNPs, etc.) in ASD through the use of next-generation sequencing technologies (NGS) is that the methods involve variables at a number of levels in the analysis that are subject to corrections from increasingly optimized mathematical programs and algorithms for analysis, which of course necessitates the need for them to be further validated experimentally.

5. Characteristics and evaluation of the dissertation and contributions

Asst. Prof. Tihomir Vachev knows in detail the current state of the problem and the extensive literature on the subject. In the introduction of the dissertation, Vachev enters into the problems to which the presented scientific development is directed, emphasizing their importance and the need to develop the dissertation topic. The text contains numerous tables and graphs that create clarity and informativeness of the survey data. The study of the literature goes from the basic to the specific aspects of the scientific issues on the topic of the dissertation. Competently considered issues, including important information regarding the issues addressed in the presented dissertation.

With regard to the formulated contributions from the dissertation, I would like to express my opinion that the contributions of a scientific-fundamental nature are of an original nature but also remain open for further research in the field. That is, they provide an opportunity for the work to continue and develop in different specific directions from the research, which I consider an additional positive side in the presented dissertation.

With regard to the formulated contributions of scientific and methodological nature, the methodological approaches used fit very precisely in the context of scientific development and the specifics of the studied neurodevelopmental disorders. I consider significant the contributions of scientific and methodological nature as they practically reveal certain searches formulated by the dissertation as different goals in the areas in which the research was conducted, namely genomic: "New generation sequencing technology has been

successfully applied" Whole-Exome Sequencing "For analysis of genetic variability in the coding regions of genes in patients with ASD", transcriptome in patients diagnosed with ASD. As well as "Digital Gene Expression Tag Profiling (DGE)" to identify differentially expressed protein-encoding genes in patients diagnosed with schizophrenia. As well as the application of proteomic analyzes "Isobaric Tag for Relative and Absolute Quantification (ITRAQ)" to study the proteomic profile in patients diagnosed with ASD.

6. Evaluation of the publications and the personal contribution of the author

The list of publications attached to the procedure for obtaining the scientific degree "Doctor of Science" includes 9 publications in Bulgarian and international journals. The quarters of the publications are reported according to the metrics of the scientific journals referred to the Scopus Scimago Journal Rank (SJR) https://www.scimagojr.com/journalrank.php. Where two of the publications presented by the Asst. Prof. Vachev are with Q2, four with Q3, and two with Q4. One of the journals (Biodiscovery) in which Asst. Prof. Vachev has a publication does not have a quartile but due to its connection with the dissertation thesis the publication is presented in the list of publications. I consider the personal contribution of the author to be the main one, judging by the fact that in all of the mentioned publications Asst. Prof. Vachev is a leading author. Therefore, a major part of the dissertation's contributions is his personal work.

7. Summary

Summaries with their content accurately reflect and reveal the main moments and ideas in the dissertation. The presented summaries in Bulgarian and English present the most important results of the study. Summaries have been prepared in accordance with the relevant requirements of the Law for development of the academic staff in the Republic of Bulgaria (ZRASRB). The Regulations for application of ZRASRB and the respective Regulations of Plovdiv University "Paisii Hilendarski".

8. Recommendations for future use of dissertation contributions and results

The dissertation thesis of Asst Prof. Tihomir Vachev is a truly large-scale scientific study that has indisputable and significance for the theory's scientific merits. The author could consider this dissertation to be published in the form of a book on the basis of a defended dissertation thesis. Future research in the field could focus on the study of the many genes (and biological pathways in which they participate) that are regulated by the miRNA molecules investigated in the studies, which are involved in neurological diseases and mental disorders. The created collection of DNA samples from patients diagnosed with ASD and schizophrenia, as well as their respective cohorts of healthy individuals represents a significant contribution of an applied nature, providing an opportunity for large-scale genetic studies in the respective field.

CONCLUSION:

The dissertation thesis "Comparative genomic, transcriptomic and proteomic studies in neurodevelopmental disorders" is in-depth and original research with a fundamental and innovative character. Scientific work has very high scientific contributions and meets all the requirements of the Law for the development of the academic staff in the Republic of Bulgaria (ZRASRB).The Regulations for application of ZRASRB and the respective Regulations of Plovdiv University "Paisii Hilendarski".

In view of the above-mentioned, as well as due to my personal impressions and research qualities of the Asst Prof. Tihomir Vachev, I give my positive assessment of the research conducted in the presented dissertation thesis. I confidently suggest to the esteemed scientific jury to award the scientific degree "**Doctor of Science**" to Tihomir Iliev Vachev, Asst. Prof. at the University of Plovdiv - Paisii Hilendarski, Faculty of Biology, Department of Plant Physiology and Molecular Biology in the field of higher education: 4 "Natural Sciences, Mathematics, and Informatics" Professional field: 4.3. "Biological Sciences" Scientific specialty: "Molecular Biology".

10. 01. 2021.   Prepared the opinion:……………………..

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