To the Chairman of the Scientific Jury

Appointed by Order No P-109-133/05.04.2024

of the Rector of the Medical University of Varna

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STATEMENT

By Prof. Ivan Yankov, MD, PhD

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of a PhD Thesis for the awarding of the educational and scientific degree "Doctor of Philosophy" under Higher education field 7 "Health Care and Sports", 7.1. "Medicine", and Ph.D. program "Pediatrics".

Ph.D. student on regular training

Ph.D. candidate: Margarita Ivanova Nikolova MD
Department of Pediatrics, Medical University of Varna
Topic: „**Genotype-phenotype correlation in patients with cystic fibrosis**"

Scientific mentors:

Prof. Miglena Dimitrova Georgieva, PhD

Prof. Gergana Petrova Stoyanova, PhD

1. General presentation of the procedure and the PhD student

The set of materials and documents presented by Dr. Margarita Ivanova Nikolova complies with the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and the Rules for its Application. It contains all the required documents.

The opinion is prepared based on the dissertation work, the author's abstract to it and the presented publications related to the topic. The volume of the dissertation is 137 typewritten pages and is distributed as follows: introduction, review of literature, background, aim and

objectives, materials and methods, results, discussion, conclusions, contributions, conclusion, appendices and literature sources. The sections are well balanced, according to the requirements for the preparation of the PhD-thesis. It contains 137 standard pages and includes 32 figures, 16 tables and 1 appendix. It is structured in 10 chapters – introduction, review of the literature, background, aim and objectives, materials and methods, results, discussion, conclusions, contributions, conclusion, appendices and literature sources, and declaration of originality. The references covers 285 sources, among which 16 are in Bulgarian and 269 in English, with the majority of sources published in the last 10 years, which I consider sufficient for a dissertation work. The abstract reflects the main data presented in the dissertation work. After the complete reading of the materials, one is left with the impression of a completed, competently, thoroughly written dissertation work.

2. Biography of the PhD student.

Dr. Margarita Ivanova Nikolova graduated her medical education in 2017 at the Medical University of Varna. She speaks English and Spanish languages.

3. Relevance of the topic of the thesis.

The PhD-thesis is dedicated to an extremely relevant problem for childhood - geno-phenotypic correlation and clinical manifestations in children with cystic fibrosis.

The PhD-thesis brings to the fore the issue of the clinical heterogeneity of the disease and the multiple gene defects that can lead to disease manifestations. Genotype-phenotype correlations in cystic fibrosis patients are the subject of a number of studies and are important not only for the diagnosis but also for the prognosis of the disease. Phenotypic manifestations depend on the percentage of normally functioning CFTR and vary from “classic” CF with exocrine pancreatic insufficiency, pulmonary involvement, high sweat test levels and obstructive azoospermia, to involvement of a single system – e.g. obstructive azoospermia, due to congenital bilateral absence of the vas deferens.

The need for a systematic diagnostic approach for timely and accurate identification of the disease was noted. A developed diagnostic algorithm is presented, which allows the diagnosis of cystic fibrosis, before the occurrence of chronic changes in the body. The dissertation draws attention to the need for precise diagnosis, which requires advanced laboratory studies, including genetic tests. Understanding the genetic basis and clinical presentation of cystic fibrosis not only allows for accurate diagnosis, but also allows guidance to optimize treatment and predict long-term outcomes.

One of the optimal approaches, indicated by the dissertation, is the knowledge of genotype - phenotype correlations in CF is useful both for predicting the course of the disease and prevention of complications, as well as for diagnosis by searching for an underlying genotype based on alarming phenotypic manifestations.

4. Literature review.

The literature review is made up of 11 parts, two of which have subsections - starting from 1/ first assumptions about MV; 2/ Historical data; 3/ Epidemiology; 4/ Cystic fibrosis mutations in CFTR; 5/ Classes of mutations in CFTR; 6/ Pathogenesis of organ damage in cystic fibrosis; 7/ Phenotypic manifestations in CF; 8/ Genotype-phenotype correlations in CF patients; 9/ Influence of gene modifiers on clinical manifestations; 10/ Diagnostic algorithm for CF; 10. 2/ Alarming phenotypic manifestations in cystic fibrosis; 10. 3/ Sweat test; 10. 4/

Genetic analysis; 11/ Progress in CF treatment 11.1 Symptomatic therapy; 12.2/ Gene-modifying therapy in MV.
From the overview, it is clear that first and second class mutations, as a rule, because a more severe clinical picture, as genotype - phenotype correlations are more demonstrative of symptoms from the GIT side. Despite the vast number of studies, a definite relationship between genotype and pulmonary phenotype could not be established. The severity of lung involvement is a determinant of morbidity and mortality in CF patients. Variability of phenotypic manifestations is determined by the influence of genetic factors, environment and adherence to therapy and is difficult to predict.

Each of the manifestations of cystic fibrosis on the part of the digestive and respiratory systems, as well as rare manifestations, have been analyzed in detail. Each of the diagnostic methods is reviewed and discussed, with an emphasis on sweat testing, genetic testing, and warning signs. Modifiers that influence gene expression are also reviewed

Based on the review, it is concluded that despite the success of gene-modifying therapy, it is still unavailable in some regions, its price is very high, and not all patients meet the criteria for treatment with it. Research continues on other corrective strategies, as well as on gene therapy to improve vector efficacy in patients with mutations not amenable to treatment with available drugs. Besides expanding the therapeutic spectrum, a leading research priority is "reducing the burden of treatment". The focus is mainly on inhaled therapy during treatment with CFTR-modulators, with a number of studies currently evaluating the real-world effects of stopping mucoactive agents. The role of physical therapy and whether it can be replaced by sports in cystic fibrosis patients is also discussed.

Thanks to the collaborative way of working and the successes achieved in the management and therapy of patients with cystic fibrosis, they are getting closer to their desired "life without limitations".

5. Aim and objectives.

The objective is clearly defined, based on the described prerequisites and the defined working hypothesis. 5 tasks with 3 sub-tasks are set, which follow the objective, allowing a good implementation of the planned study. The tasks cover the research design, which is well chosen.

6. Material and methods.

At the beginning of this section, the design of the study is described - a retrospective study using the information for 45 patients from North-Eastern Bulgaria with a diagnosis of CF, who are being followed up and treated in the Reference Center at the UH "St. Marina". A period of 5 years is covered - from January 2019 to December 2023. Inclusion and exclusion criteria are specifically and clearly described.

Clinical, laboratory and statistical methods were used that were appropriate and appropriate to the purpose and tasks.

The cohort of patients is sufficient to obtain reliable results, which are analyzed with appropriately selected statistical methods and illustrated with tables and figures. The material is sufficient to produce reliable results. The setting of the study, the stages of the study and the research methods used are well described.

7. Results and discussion.

The PhD thesis analyzed data of 45 patients with proven cystic fibrosis. The sample represents nearly 25% of the patients in Bulgaria. After consent from the patients to participate, the information is submitted either from national registries or from local centers. The participation of cystic fibrosis patients from North-Eastern Bulgaria allows monitoring the progress and treatment of the disease, promotion of new standards of care, acquisition of epidemiological information to improve the planning of interventions in the field of public health.

A predominance of the male gender was noted for North-Eastern Bulgaria. The percentage distribution by ethnicity of cystic fibrosis patients from Northeast Bulgaria also differs from the distribution for Bulgaria. In the distribution of cystic fibrosis mutations, it is not surprising that the most common for the patient group studied was F508del.

Neonatal screening for cystic fibrosis was not available at the time of the study. In conditions of missing one, early recognition of alarming symptoms is relied upon to confirm the diagnosis of cystic fibrosis

A significant correlation was also found regarding the decrease in FEV1 and the increase in the age of the patients, confirming the progression of the disease over time. And while before end-stage lung disease put cystic fibrosis patients on the list for lung transplantation, with the introduction of CFTR-modulating therapy, this step is taken less frequently, not only because of the delay in progression, but also because of the significant improvements in lung function.

Based on the results, it is concluded that the role of genotype-phenotype correlations in CF patients is important for predicting the course of the disease, as well as for searching for the underlying genotype in certain phenotypic manifestations and timely diagnosis.

The detailed presentation of the results is impressive, as the discussion is directed and competent, following point by point the basis of obtained results. It shows the skill of Dr. Margarita Nikolova to analyze her own results in the context of the data known in the literature. The author compares his results with the results of known studies on the subject.

8. Conclusions

Based on the obtained results, 5 conclusions are formulated, which follow the logically set aim and objectives of the study.

9. Author's summary of Ph.D. Thesis

The presented author's summary has a volume of 60 pages and reflects the content of the thesis and the requirements.

9. Publications related to the thesis

A totally 5 articles are presented. In terms of number and quality, the scientific works exceed the minimum requirements for the scientific degree " Doctor of Philosophy ", according to the Regulations of the Medical University of Varna.

10. Participation of the PhD student

The thesis, the contributions and the obtained results are the personal work of the PhD student.

11. Critical remarks and recommendations.

None.

12. Personal impressions

I have not a personally known Dr. Margarita Nikolova. With this PhD thesis, Dr. Margarita Nikolova demonstrates the qualities of an established thorough researcher.

13. Recommendations for future use of dissertation contributions and results

I recommend Dr. Margarita Nikolova to present the established results relevant to practice in a monographic publication.

CONCLUSION

The PhD thesis contains scientific results that represent an original contribution to science and meet all the requirements, according to the regulations for the terms and conditions of acquiring scientific degrees and obtaining academic degrees at the Medical University of Varna, the Act for the Development of the Academic Staff in the Republic of Bulgaria, and the Regulations for its application.

The PhD thesis shows that Dr. Margarita Nikolova possesses theoretical knowledge and professional skills in the scientific specialty by demonstrating qualities for conducting scientific research

I recommend to the respected scientific jury to vote positively and to award Dr. Margarita Ivanova Nikolova the educational and scientific degree "Doctor of Philosophy" under Higher education field 7 "Health Care and Sports", 7.1. "Medicine", and Ph.D. program "Pediatrics".

29.05.2024 г. Reviewer

Prof. Ivan Yankov, MD, PhD