To

The Chairman of the Scientific Committee,

as determined by Order No. R 109-133/05.04.2024

by the Rector of the Medical University - Varna

Attached herewith:

REVIEW

by Prof. Dr. Miroslava Nikolova Bosheva, MD, Department of Pediatrics, Medical University - Plovdiv

on a dissertation submitted for the award of the educational and scientific degree of 'Doctor'

in the professional field of Pediatrics 03.01.50.....

doctoral program in Pediatrics 03.01.50 ..

Author: Margarita Ivanova Nikolova...

Form of doctoral studies: full-time doctoral student at the Department of Pediatrics, Medical University - Varna

Department: Pediatrics, Medical University "Prof. Dr. Paraskev Stoyanov" Varna

Topic: GENOTYPE - PHENOTYPE CORRELATION IN PATIENTS WITH CYSTIC FIBROSIS

Academic supervisors: Prof. Dr. Miglena Georgieva, MD, and Prof. Dr. Gergana Petrova, MD

I declare that I have no joint publications with the doctoral student.

1. General presentation of the procedure and the doctoral candidate

The submitted set of materials in paper/electronic format complies with Article 69(1) of Part I. Acquisition of educational and scientific degree "DOCTOR" and scientific degree "DOCTOR OF SCIENCES" at MU-Varna; Regulation of MU-Varna dated November 22, 2022, and includes the following documents:

1. Application to the Rector to initiate a defense procedure;
2. Curriculum vitae in European format signed by the doctoral candidate;
3. List of publications related to the topic of the dissertation work signed by the doctoral candidate;
4. Copies of publications related to the topic of the dissertation work;
5. Declaration of authenticity of the submitted documents;
6. Abstract in Bulgarian language, formatted according to the requirements of MU-Varna, and in English language (on electronic media - flash drive);
7. The doctoral candidate has provided copies of 5 publications.

2. Brief biographical data about the doctoral candidate

Dr. Nikolova obtained her higher education at MU Varna in 2017, and immediately after graduation, she began specializing in pediatric pulmonology at the 2nd Pediatric Clinic of University Hospital "St. Marina" in Varna. Since 2019, she has been enrolled as a regular doctoral student at the Department of Pediatrics - MU Varna, and since February 2023, she has been appointed as an assistant professor at the same department. She is proficient in English and Spanish languages.

3. Relevance of the topic and appropriateness of the objectives and tasks

I find the chosen topic of the dissertation to be highly relevant to the present day, considering the need for timely diagnosis of children with cystic fibrosis and, most importantly, the clarification of their genetic defect to guide treatment decisions aimed at improving their survival and quality of life. The objective is formulated broadly, but nevertheless, the five tasks for achieving it are logical and specific.

4. Understanding of the problem by the candidate

The dissertation consists of 137 pages and includes 16 tables, 32 figures, and 2 appendices. Upon reading the dissertation, one gets the impression of a deep understanding of the problem. The literature review is intelligently written, following the logic of the research. It spans 40 pages and is based on 285 literature sources, including 16 in Cyrillic. It is divided into 13 chapters covering historical data, epidemiology, classes of mutations in CFTR, pathogenesis of organ damage in cystic fibrosis, phenotypic manifestations, genotype-phenotype correlations in CF patients, the influence of gene modifiers on clinical manifestations. Additionally, the diagnostic algorithm for CF, alarming phenotypic manifestations in cystic fibrosis, progress in CF treatment, and the future for CF patients are discussed. Assumptions for choosing the topic of the dissertation are derived from the analysis of the literature sources.

5. Research methodology

The chosen research methodology allows for achieving the set objective and obtaining an adequate response to the tasks addressed in the dissertation. The study includes 45 patients aged 5 months to 37 years with confirmed CF who are enrolled in the National Cystic Fibrosis Registry and are followed up at the Cystic Fibrosis Center at University Hospital "St. Marina" – Varna. They were selected based on precise inclusion and absence of exclusion criteria. The nature of the study is retrospective, covering a 5-year period from January 2019 to December 2023. Perhaps the characteristics of the cohort – gender, age, and ethnic distribution – should have been described in the dissertation material.

Data from anthropometric measurements were analyzed every 3 or 6 months, depending on the patient's age, as well as microbiological studies of the respiratory tract, sweat test to assess treatment response, FEV1, imaging studies (chest X-ray, CT scan, abdominal ultrasound). All information was processed using modern statistical methods. I find the material and research methods sufficient for obtaining real results and addressing the research objectives.

7. Characteristics and evaluation of the dissertation

The dissertation has two very strong aspects:

1. Genotype-phenotype correlation regarding lung involvement (presence of bronchiectasis, FEV_1, chronic Pseudomonas colonization, nasal polyposis); gastrointestinal manifestations (BMI, pancreatitis, meconium ileus), sweat test

values, diabetes, infertility, providing valuable insights for clinicians to outline the prospects for the affected child's health.

2. Development of an algorithm for early CF diagnosis, which will find application in clinical practice regardless of the upcoming introduction of neonatal screening. This will reduce the average age of diagnosis from 2.14±5.32 years (result for Northeast Bulgaria) to the neonatal period.

Based on excellent statistical analysis of the results, the author draws conclusions that I find acceptable, namely:

1. Patients with genotypes including mutations from class I or class II exhibit more severe phenotype manifestations in the respiratory system compared to patients with genotypes including mutations from classes III – VI or VUS. The G542X mutation from class I shows milder phenotype manifestations concerning the pulmonary phenotype compared to other mutations investigated from the same class and compared to F508del.

2. Patients with genotypes including mutations from class I or class II exhibit more severe phenotype manifestations in the gastrointestinal tract (GIT) compared to patients with genotypes including mutations from classes III – VI or VUS. Patients with the genotype F508del/F508del have an average malnutrition status. The G542X mutation from class I shows more severe phenotype manifestations concerning BMI and CFLD compared to other mutations investigated from the same class and compared to F508del.

3. Patients with genotypes including mutations from class I or class II have higher sweat test values compared to patients with genotypes including mutations from classes III – VI or VUS. The G542X mutation from class I is characterized by lower sweat test values compared to other mutations investigated from the same class and compared to F508del. CFRD is reported only in patients with the genotype F508del/F508del.

7. Contributions and significance of the dissertation for science and clinical practice.

The contribution of the dissertation is significant both for science and for practice in the field of cystic fibrosis. The author effectively demonstrates the importance of early diagnosis and individualized treatment for patients with this rare genetic disease. The proposed algorithm for early diagnosis can serve as a valuable tool for clinicians in clinical practice, which may contribute to improving the survival and quality of life of affected patients. Such research is of paramount importance for the advancement of medicine, and we hope that the results of this research will be reflected in future medical practices and guidelines.

8. Evaluation of the publications related to the dissertation work.

Dr. Nikolova provides 5 publications related to the dissertation work, out of which 3 are in peer-reviewed journals. In all publications, Dr. Nikolova is the first author.

9. Personal involvement of the doctoral candidate in the reseach.

Reading through the dissertation, it is apparent that the data analysis from the 45 patients at the Cystic Fibrosis Center at University Hospital "St. Marina," Varna, and the data from the European Cystic Fibrosis Society Patient Registry (ECFSPR) is the personal work of the doctoral candidate. Following thorough analysis, Dr. Nikolova has drawn results, conclusions, and contributions, with the most significant being the development of a practical algorithm based on alarm symptoms for cystic fibrosis. This aims to facilitate early referral to a specialized center for further diagnosis, treatment, and follow-up.

10. Abstract

The abstract submitted fully meets the requirements for such a publication in terms of content, quality, and formatting. It clearly outlines the purpose, objectives, methods, and results of the conducted study. The conclusions and contributions are clearly delineated. The abstract has a very good aesthetic appearance.

11. Critical points and recommendations

Based on the thorough review of the dissertation and associated materials, I have identified several critical points and recommendations: As in the dissertation itself as well as in the abstract, the Scientific Field of Higher Education: 7. Healthcare and Sports, Professional Field: 7.1. Medicine, and Scientific Specialty: 03.01.50 Pediatrics are not mentioned.

12. Personal Impressions

Unfortunately, I do not know the doctoral student, but I am very familiar with the intellectual and professional abilities of his supervisors, who would not mentor a young doctor if she did not possess the necessary scientific skills for a doctoral student.

13. Recommendations for Future Use of Dissertation Contributions and Results

I consider it appropriate for the proposed algorithm for early disease recognition to become not only a part of pediatric literature but also to be included in printed publications intended for general practitioners in Bulgaria.

CONCLUSION

The dissertation contains scientific, scientific-applied, and applied results that represent an original contribution to science and meet all the requirements of the Law on the Development of Academic Staff in the Republic of Bulgaria (LDASRB), the Regulation for the Implementation of LDASRB, and the respective Regulation of MU - Varna. The materials and dissertation results presented fully comply with the specific requirements of MU - Varna.

The dissertation demonstrates that the doctoral student Dr. Margarita Ivanova Nikolova possesses in-depth theoretical knowledge and professional skills in the scientific specialty of pediatrics, demonstrating qualities and skills for independent scientific research.

Therefore, based on the above, I confidently give my positive assessment of the conducted research, presented by the reviewed dissertation, abstract, achieved results and contributions, and I recommend to the esteemed academic jury to award the educational and scientific degree 'Doctor' to Dr. Margarita Ivanova Nikolova in the doctoral program in pediatrics.

10 May 2024

Prof Miroslava Bosheva, MD, PhD